STUDIES IN THE TRANSDUCTION OF A LARGE F-PRIME, F14, BY BACTERIOPHAGE P1*kc*

By

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Previous genetic evidence has indicated the presence of "F14-like" merogenotes in transductants made with P1 grown on AB1206, an F-prime strain haploid for the region carried on the F14. Here we report physical and biochemical evidences, which taken together demonstrate that the transduced plasmids are indeed indistinguishable from F14. Three size classes were found: 3.3 times [311 kilobases (kb)], 2.3 times (217 kb), and 1.0 times (94.5 kb) the size of F. Heteroduplex analysis showed several structures present on the transduced F14s that are also part of the F14 structure. They have all of the F sequence (94.5 kb) which includes the αβ directly repeated sequence (IS3) of F. They also have another directly repeated sequence, which occurs at the two junctions of F DNA with chromosomal DNA. There is another directly repeated sequence that measures 5.2 kb

(*rrnA* and *rrnB*), which has a small 0.5 kb non-homology bubble (the *rsp* spacers).

Heteroduplex analysis also revealed that the 1.0 times F size class molecules in transduced F14 strains are F. This is consistent with those found in the parental F14 strain's plasmid population. The F has been proposed to form by intermolecular recombination events between the sequences. Gel electrophoretic analysis of *Eco*R1 fragmented plasmid DNA showed that transduced F14s and parental F14 have identical gel patterns, sharing 44 bands (fragments greater than 0.38 kb in size), indicating that their sequences are identical. Comparisons of gel patterns with F and F*ilv*s were used to identify the F. sequences, the *ilv* sequence and chromosomal sequences. Both F14s also showed bands with identical molecular weight as the fragments previously identified for the *ppc-arg*ECBH-*bfe* chromosomal region. These data, taken together, support the genetic evidence that the "F14-like" plasmids in P1 transductants are indistinguishable from F14.

The mode of genetic transfer of the F14 in these P1 lysates is transduction. This is a seemingly impossible transduction since the F14 would appear to be too large (205 x 10^6 daltons) to be transduced by P1 (whose normal transducing particles carry 64 x 10^6 daltons); however, the frequency of transduction of the F14 (7 x 10^{-8}) is too high to be transduced by multiple infection of transducing particles carrying complementary fragments. A dose-response curve supports the model of one transductional unit. Further studies revealed that the unit is probably one transducing particle. Due to the amount of DNA transduced, the transducing particle should be larger and/or more dense. Such phage particles were not found by cesium chloride

density centrifugation, further, the capability of P1 to transduce the F14 is lost during the centrifugation. A model is proposed for the transduction of F14 by one particle.

PART I

MOLECULAR AND BIOCHEMICAL EVIDENCE FOR F14S IN TRANSDUCTANTS MADE WITH BACTERIOPHAGE P1 FROM AN F14 MEROGENOTE OF *ESCHERICHIA COLI* K 12

Introduction

P1 virulent particles carry a complement of linear deoxyribonucleic acid (DNA) of 64 x 10^6 daltons or 97 kilobase pairs (kb) (Ikeda and Tomizawa, 1965; Lee, Ohtsubo, Deonier and Davidson, 1974; Backmann, Low and Taylor, 1976). Transducing particles carry a fragment of host chromosomal DNA which usually has the same molecular size as the genome of the virulent particles (Ikeda and Tomizawa, 1965; Lee *et al.*, 1974; Rae and Stodolsky, 1974; Rosner, 1975; Bachmann *et al.*, 1976). But, there have been several cases reported that suggest that P1 has transduced F-primes that are larger than the DNA complement carried by normal transducing particles, *e.g.* F8 (117 kb) and F14 (311 kb) (Ohtsubo, 1971; Pittard and Adelberg, Bacteriol. Proc., p. 138, 1963). The case which we have been examining in detail is the putative transduction of F14. This very large F-merogenote is transduced at a frequency of 7.0 x 10^{-8} per plaque forming unit (p.f.u.) (Hendrickson and Duggan, 1976). The transduced molecules are genetically indistinguishable from the parental F14 in terms of (a) easily detectable genes transferred into *rec*A recipients (*ilv*EDAC, *met*E, *rha*, *met*B, *arg*H), (b) the order of transfer of genetic markers, and (c) the genetic distance (time-of-entry) between proximal and distal markers (*met*B and *ilv*D)

(Hendrickson and Duggan, 1976). The genetic evidence obtained suggests that a molecule more than three times the size (311 kb) of that normally carried by P1 has been transduced. Whether by one particle or several, the mechanism of such a seemingly unlikely transduction is of great interest; but, since other explanations could be proposed to fit the genetic data, firm physical and biochemical evidence for the presence of F14 plasmids in transductants is needed before studies on the mechanisms are justified.

The focus of the research reported here is to present the physical and biochemical evidence for the presence of F14 plasmids in the transductants. The identity of the physical structures of the transduced F14s is determined by contour measurements of DNA molecules on electron micrographs and by DNA heteroduplex analysis; the biochemical identities of the molecules are determined by examining gel electrophoresis patterns of restriction fragments.

Materials and Methods

Media.

All strains are routinely grown in Luria (L) broth (Luria and Burrows, 1957) when a complex broth medium is required and with the addition of 2.0% agar for complex plating medium. The Z broth (L broth with 2.5×10^{-3} M $CaCl_2$) (Luria and Burrows, 1957) is used to grow both the donor and recipients in transductions where P1*kc* is used as a vector. The Z agar (Z broth with 1% agar) is used as a bottom agar for making and titering phage lysates. The SA-1 agar (0.7% agar and 1.0% NaCl) is used as an overlay agar on Z plates for plaque forming assays and for making lysates on plates (Hendrickson and Duggan, 1976). Half-strength medium 56 (Adelberg and Burns, 1960) is a minimal medium routinely used for the selection of recombinants and for the growth of F-merogenote strains to prevent segregation of F-primes used in the study. All amino acids, purines and pyrimidines are supplemented as required in 56/2 at final concentrations as described in Hendrickson and Duggan (1976).

Bacterial Strains.

The bacterial strains used are described in Table 1. The haploid F14 strain, χ1254 (isolated from AB1206 in the laboratory of Roy Curtiss III) (Pittard, Louitt and Adelberg, 1963; Ohtsubo, Deonier, Lee and Davidson, 1974a; Hendrickson and Duggan, 1976) is used as the donor strain in transduction experiments. It is also used as

Table 1. *Escherichia coli* K12 Strains

Strain	Sex	Genotype	Source or Derivation
χ1254 (AB1206)	F14	(F-*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)Δ(min 83.2 to 88.1) *thi*-1, *his*-1, *pro*A2, *lac*Y1, *trf*-3, *str*	Eiichi Ohtsubo State University of New York at Stony Brook
AB2989	F16	(F-*ilv*EGDACB)/*ilv*E12, *arg*G12, *his*42	This laboratory[a]
AB2988	F310	(F-*ilv*EGDACB)/*ilv*E12, *arg*G12, *his*42	This laboratory[a]
AB2997	F316	(F-*ilv*EGD)/*ilv*E12, *arg*G12, *his*42	This laboratory[a]
JC411	ColEI	*thy*$^-$	Daniel Vapnek University of Georgia
AB264	F$^+$	F$^+$/*ara*$^{(mu-1)}$, λ$^+$	This laboratory
W6	F*ins*58.8	F$^+$*ins*58.8/*met*B	Eiichi Ohtsubo
AB1450	F$^-$	*ilv*D16, *met*B1, *arg*H1, *his*-1, *str*, *tsx*[b]	Barbara Bachmann Yale University
AB1472	F$^-$	*ilv*D16, *met*B1, *arg*H1, *str*[c]	Barbara Bachmann
KF101	F$^-$	As AB1472, also *rec*A1	This laboratory[d]
KF104	F$^-$	As AB1472, also *rec*A1	This laboratory[d]
KF2201	F$^-$	*thi*-1, *met*E46, *trp*-3, *his*-4, *str*, *rha*	This laboratory[d]
KF105	F$^-$	As AB1472, *nal*A	This laboratory[d]

Table 1. Continued

Strain	Sex	Genotype	Source or Derivation
KF636	F14(P36)	(F-*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)/AB1450	P1·χ1254 x AB1450
KF732	F14(P132)	(F-*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)/AB1450	P1·χ1254 x AB1450
KF733	F14(P133)	(F-*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)/AB1450	P1·χ1254 x AB1450
KF436	F14(P36)	(F-*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)/KF104	KF636 x KF104
KF532	F14(P132)	(F-*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)/KF104	KF732 x KF104
KF533	F14(P133)	(F-*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)/KF104	KF733 x KF104

[a] Derivation in N.J., Marsh and D.E. Duggan (1972)

[b] This strain also carries *thi*-1, *xyl*-7, *mal*A1, *lac*Y1, *gal*-6, *ton*A-1, λ^r, λ^-

[c] This strain also carries *thi*-1, *mal*A1, *gal*-6, λ^r, λ^-

[d] Derivation in E.R. Hendrickson and D.E. Duggan (1976).

the source of F14 plasmid DNA in contour measurements of molecules, heteroduplexing analysis and restriction enzyme mapping. Each "F14-like" merogenote strain in derived as previously described (Hendrickson and Duggan, 1976). The "F14-like" *recA* strains are used in time-of-entry (T.O.E.) matings and as a source of plasmid DNA.

Bacteriophage

The stock of P1*kc* (Lennox, 1955) was obtained from Roy Curtiss III (University of Alabama in Birmingham).

Production of Phage Lysates

All P1*kc* lysates are made by the soft-layer method (Swanstrom and Adams, 1951), using a simplified harvesting procedure (Marsh and Duggan, 1972).

Time-of-Entry Matings

The procedure employed for time-of-entry matings is that of Adelberg and Burns (1960) with modifications as described previously (Hendrickson and Duggan, 1976) using nalidixic acid as a male counter-selecting agent.

Transduction Procedure

Procedures are the same as described previously (Hendrickson and Duggan, 1976).

Isolation of Plasmid DNAs

Plasmids were isolated using the method described by Sharp *et al.* (1972). Some of the modifications made in the procedure for isolation

of F14 and transduced F14s were determined in this laboratory and others were suggested in Ohtsubo *et al.* (1974a). Spheroplasts were lysed at 37°C not 0°C. The shearing step was either omitted or the DNA was gently sheared by a single slow passage (90 to 120 sec) through a 50 ml syringe without a needle. Both procedures gave similar concentrations of covalently closed circular (CCC) molecules. Since the lysate was very viscous, the NaOH was added very slowly during the denaturation step as suggested by Ohtsubo *et al.* (1974a) and Dean Rupp (personal communication), to allow for sufficient mixing and time for the pH meter to respond to the change in pH. Both a magnetic stirrer (1 rev/sec) and a teflon policeman were used to ensure adequate mixing. The pH was titrated to 12.2 and maintained for three to five minutes. The sodium ion concentration was adjusted to 0.3 M for the most effective absorption of single-stranded DNA on nitrocellulose (Hercules, 1/4 sec). After the nitrocellulose step and removal of nitrocellulose by centrifugation, the lysate was filtered through glass wool. This was repeated again, after the DNA was pelleted into the CsCl shelf. Both steps remove debris that would interfere with the CsCl/ethidium bromide (EthBr) banding.

In the dye-buoyant density centrifugation, the density was adjusted to 1.57 g/cc with CsCl and EthBr was added to a final concentration of 500 µg/ml. The DNA was banded using a Beckman Type 40 rotor for 36 hr at 35 krev/min. Since gentle shearing or no shearing has been used, the upper band (linear and open circled DNA) was broad and viscous and had to be removed with a capillary micropipet because it interfered with the collection of the lower band by dripping. The Type 40 rotor was also used in rebanding

of the pooled bands from the first run. The DNA is stored in the dark at 4°C in the CsCl/EthBr solution.

Electron Microscope Methods

The basic protein film technique of Kleinschmidt (1968) was used for mounting DNA samples for examination in the electron microscope. Using the aqueous technique (Davis, Simon and Davidson, 1971), the DNA was examined for contour size measurements and homogeniety. ColEI (JC411) and F (which is naturally present in F14 samples (Ohtsubo *et al.*, 1974a)) were used as internal standards. Since all of the plasmids were isolated in EthBr, it was unnecessary to remove the dye.

The formation of heteroduplex molecules of transduced F14s and F-prime plasmids of known sequences was carried out using the alkaline-formamide technique (Davis *et al.*, 1971; Sharp *et al.*, 1972; Ohtsubo *et al.*, 1974a). Approximately 0.1 µg of each species of DNA (ranging in concentration from 10 µg to 100 µg/ml depending on plasmid used) were added to the heteroduplex mixture. The heteroduplex mixture consisted of 20 µl of 1 N NaOH plus the DNA samples and double distilled H_2O to 80 µl. The mixture was allowed to stand four min before it was reneutralized with 20 µl of 1 M Tris-HCl and 100 µl of 0.2 M EDTA, pH 8.5. The DNA mixture was renatured in the presence of 70% formamide under the conditions used by Sharp *et al.* (1972).

Heteroduplexes were mounted by using the formamide technique (Davis *et al.* 1971; Sharp *et al.*, 1972). ColEI was used as the dsDNA length standard (6.34 kb) and ΦX174 was used as the ssDNA length standard (5.375 kb).

The heteroduplexed molecules were measured by a Numonics Graphics length calculator. The contour lengths of covalently closed circular molecules, enlarged on translucent paper were determined by a Dietzgen Plan Measure. Electron micrographs were made using either a Hitachi HU-11C electron microscope or a Phillips EM-201 electron microscope.

Restriction Endonuclease Fragmentation and Gel Electrophoresis

For *Eco*R1 restriction nuclease cleavage one must first remove the EthBr and concentrate the DNA (sample contained 10 to 20 µg/ml DNA) (E. Ohtsubo, personal communication). The EthBr was removed by five equal volume extractions with isopropanol equilabrated with a saturated CsCl solution (the aqueous phase will contain the DNA). The DNA sample (500 µl) was concentrated to 100 µg/ml by ethanol precipitation, 3 parts 100 % ethanol to 1 part DNA solution. The mixture was incubated in an ethanol-dry ice bath for ten min. The ethanol-DNA solution was centrifuged for ten min at 10 krev/min. The supernatant was poured off and the DNA pellet was resuspended in 100% ethanol and reincubated in the ethanol-dry ice bath for ten min. The ethanol-DNA was centrifuged for ten min at 10 krev/min. All but approximately 0.1 ml of ethanol was poured off. The remaining ethanol was removed by evaporation on a lyophilizer. The remaining DNA pellet was resuspended in TE buffer (0.01 M Tris, 0.001 M EDTA, pH 7.2).

The restriction endonuclease, *Eco*R1 (1.4×10^5 units/ml, Miles Laboratories), cleavage of plasmid DNA was carried out using a modification of Greene *et al.* (1974). The incubation period was two hr at 37°C and the final DNA concentration was 80 µg/ml.

The method of Sharp *et al.* (1973) was used for agarose gel electrophoresis of *Eco*R1 plasmid DNA fragments. Agarose (0.5% and 0.7%) gels were made with E buffer (0.4 M Tris, 0.02 M NaOAC, 0.003 M EDTA, 0.18 M NaCl, pH 8.0). Samples were then prepared for loading onto the gel: 10 µl DNA sample, 10 µl E buffer, 4 µl dye solution (0.025% bromphenol blue and 50% glycerol in E buffer). Electrophoretic separation was accomplished by applying 100 V over 15 cm, allowing enough time for the tracking dye to run 12 cm (four to six hr).

DNA bands were visualized by fluorescence over a long wave ultraviolet light after staining the gels for one hr in E buffer containing ethidium bromide (2 µg/ml) (Sharp, Snyder and Sambrook, 1973). Gels were photographed using a short wave ultraviolet light and Polaroid 57 (ASA 3000) film or Polaroid 55 (p/n) film.

Four percent polyacrylamide gels in E buffer (4 gm acrylamide, 0.2 g N'N'-methyline bisacrylamide, 1.0 ml 10% NH_4 persulfate, 50 µl TEMED in 100 ml of E buffer) were used to resolve the smaller *Eco*R1 fragments (less than 2.5 kb). Electrophoretic separation was carried out by applying 100 V over 15 cm, allowing enough time for the tracking dye to travel eight cm (four to six hr). DNA bands were visualized and photographed using the same procedure as above.

Molecular lengths of *Eco*R1 DNA fragments were determined by plotting Rf vs the log of the molecular length (in kilobases). The ten *Eco*R1 fragments of F8(P6) and the 19 *Eco*R1 fragments generated from F were used as standards in estimating the molecular length of other DNA fragments in the same gel (E. Ohtsubo, personal communication; Childs *et al.*, 1977).

Results

Genetic Properties and Contour Measurements of F14

The F14 is an F-prime that is harbored in an *Escherichia coli* K12 strain, AB1206. This strain is haploid for all or most of the genes carried on the F14 chromosomal sequence (Pittard *et al.*, 1963; Pittard and Ramakrishnan, 1964; Glansdorf, 1976; Ohtsubo *et al.*, 1974a; Deonier, Ohtsubo, Lee and Davidson, 1974). AB1206 transfers *ilv*EDAC$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$ and *arg*H$^+$ to both *rec*A$^+$ and *rec*A$^-$ (Ohtsubo *et al.*, 1974a; Hendrickson and Duggan, 1976). The F14 carries these genetic markers and are mapped as shown in Figure 1 (Pittard *et al.*, 1963; Glansdorf, 1967; Ohtsubo *et al.*, 1974a). The bacterial sequence on F14 consists of 210.8 $\pm$ 10 kb and is indicated in the structure map (Figure 1) as sawtoothed lines. The 94.5 kb F sequence indicated in the map by smooth lines is complete on F14. However, there is a 5.7 kb sequence, $\gamma\delta$ (2.8 to 8.5 F), which occurs only once on F, this is directly repeated on the F14. This sequence occurs at each of the junctions of F DNA with chromosomal DNA which are labelled as 8.5 F/OB and 210.8 B/2.8 F, respectively. The molecular length of F14 is therefore 311 kb (Ohtsubo *et al.*, 1974a).

The F14 has another sequence, $\alpha\beta$, that is repeated twice in its F sequence [this is also true for F (Davidson, Deonier, Hu and Ohtsubo, 1975)]. They map at 93.2 to 94.5 F and 13.7 to 15.0 F. The F14 also has two ribosomal RNA gene sets, *rrn*A and *rrn*B. The

Figure 1. Structural map of F14 and its segregated subunits. Gene positions are as mapped by Ohtsubo *et al.* (1974a), Lee *et al.* (1974), Ohtsubo *et al.* (1974b), Deonier *et al.* (1974), Ohtsubo *et al.*(1974c), Bachmann *et al.* (1976) and Sharp *et al.* (1972). The F sequence coordinates are expressed in kilobase-pair units (kb) followed by the suffix F and are depicted in a clockwise order on F14. The chromosomal coordinates are also given in kb units followed by the suffix B and are depicted in a clockwise order on F14 as it is on the chromosome of *Escherichia coli* K12. Physical observations show that F14 has a tendency to segregate into F and F14ΔF (Ohtsubo *et al.*, (1974a). The structural maps of these segregated molecules are shown here.

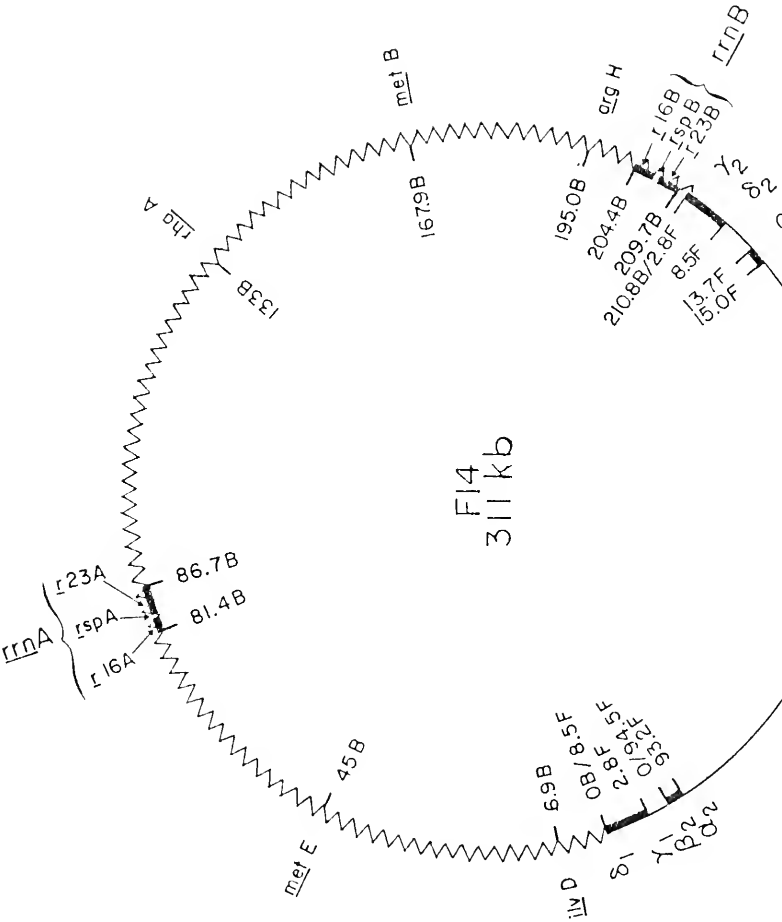
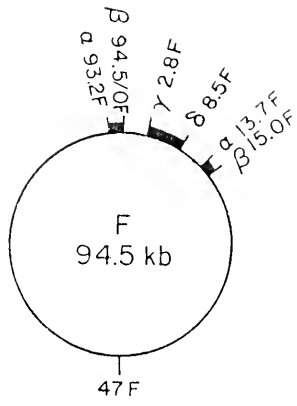
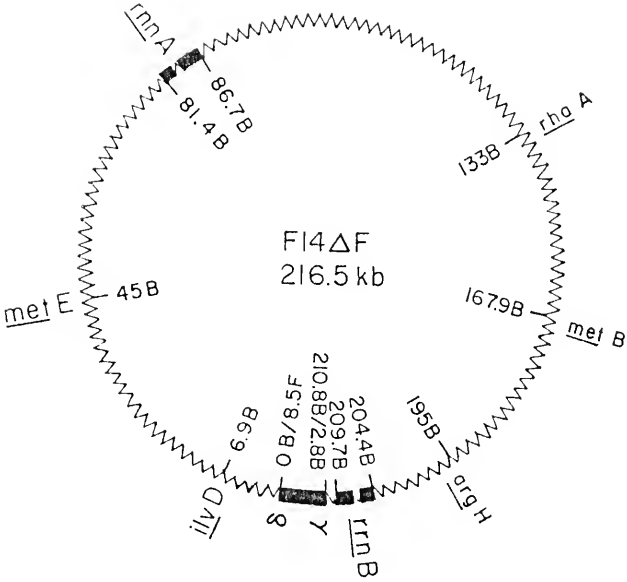

F14
311 kb
rrnA
r23A
rspA
r16A
86.7B
81.4B
45B
met E
ilv D
6.9B
0B/8.5F
2.8F
0/94.5F
93.2F
δ1
γ1
β2
α2
47F
13.7F
15.0F
8.5F
210.8B/2.8F
209.7B
204.4B
195.0B
167.9B
133B
rha A
met B
arg H
r16B
rspB
r23B
rrnB
γ2
δ2
α1
β1

Figure 1. Continued. Structural map of F14 and its segregated subunits.


β 94.5/0F
α 93.2F
γ 2.8F
δ 8.5F
α 13.7F
β 15.0F
F
94.5 kb
47F


rrn A
86.7B
81.4B
rha A
133B
F14ΔF
216.5 kb
met B
167.9B
195B
arg H
204.4B
209.7B
210.8B/2.8B
rrn B
γ
δ
0 B/8.5F
6.9B
ilv D
met E
45B

DNA sequences of *r*16A and *r*16B, and *r*23A and *r*23B are identical. The spacer sequences, *rsp*A and *rsp*B, are different (Ohtsubo *et al.*, 1974a; Deonier *et al*, 1974; Ohtsubo, Soll, Deonier, Lee and Davidson, 1974c; Davidson *et al.*, 1975).

Plasmid DNA extracts of F14 strains contain at least three classes of molecules: 3.3, 2.3 and 1.0 times the length of F. These have the respective molecular lengths of 311 kb, 216.5 kb and 94.5 kb. By heteroduplex mapping, these molecules are identified as the F14, the F14 chromosomal sequence plus one copy of $\gamma\delta$ (F14ΔF subunit), and the F sex factor, respectively (Ohtsubo *et al.*, 1974a).

It has been long observed that AB1206 will lose its donor ability for F14 markers after long storage, but retain its F characters. Since there are three plasmids, F14, F14ΔF and F, present in F14 plasmid extracts, it has been postulated that F14 segregated into the two smaller molecules, F and the F14ΔF subunit (Figure 1). The interpretation is that the instability of F14 is due to reciprocal recombination between the two $\gamma\delta$ sequences on F14. This 5.7 kb sequence has been proposed to be a hot spot for F recombination (Ohtsubo *et al.*, 1974a; Davidson *et al.*, 1975). The repeated sequences (1.3 kb) could also produce segregated molecules of similar class sizes, but recombination probably occurs at a frequency too low to be observed (Davidson *et al.*, 1975). This sequence has been shown to be active in reciprocal recombination in some hosts of F152 (Ohtsubo *et al.*, 1974a).

The genetic properties of χ1254 (genetic markers, F14 markers transferred and kinetics of transfer of F14 markers) are the same

as those described for AB1206 by other investigators (Pittard *et al.*, 1963; Pittard and Ramakrishnan, 1964; Glansdorf, 1967; Ohtsubo *et al.*, 1974a). The plasmid DNA isolated from χ1254 in this study contains three classes of molecules (Table 2); they are the same size classes as reported by Ohtsubo *et al.* (1974a), 3.3, 2.3 and 1.0 times F. Their relative frequency of occurrence in the DNA mixture is less than those reported by Ohtsubo *et al.* (1974a) because we did not use the x-ray isolation method to obtain open circles (Sharp *et al.*, 1972; Ohtsubo *et al.*, 1974a). The method of isolation of supercoiled circular molecules favored the isolation of the smaller F class molecules due to the greater probability of nicking in the larger molecules. Based on transfer kinetics, genes transferred and the contour measurements of the plasmid population we conclude that the donor F14 from χ1254 is the same as the F14 previously described by Pittard *et al.* (1963) and Ohtsubo *et al.* (1974a). Data from studies reported below do not contradict this conclusion.

Genetic Properties of Transduced F14s and Their Contour Measurements

Three *rec*A$^-$ strains, KF436, KF532 and KF533, carrying transduced F14 plasmids are used in this study. The transduced F14s in these strains are designated F14(P36), F14(P132) and F14(P133), respectively. F14(P36) in KF436 has been described previously (Hendrickson and Duggan, 1976). The other transduced F14s, F14(P132) and F14(P133), transfer F14 genetic markers (*ilv*D, *met*E, *rha*, *met*B and *arg*H) to both *rec*A$^+$ and *rec*A$^-$ strains (AB1472, KF104 and KF2201). F14 (P133)'s time-of-entry of F14's proximal marker (*met*B), and distal marker (*ilv*D) are similar to those described previously

Table 2. Size classes of circular DNA from χ1254 and the *recA1*$^-$ strains carrying the transduced F14s

	I	II	III
A. From χ1254 (F14)			
Molecular length (F units)	3.26 ± 0.06 (6)[a]	2.28 ± 0.02 (4)	1.00 (12)[b]
Molecular length (kb)	308.3 ± 6.0	215.3 ± 2.1	94.5
Frequency in sample	0.25	0.25	0.50
B. From KF436 (F14(P36))			
Molecular length (F units)	3.26 ± 0.09 (7)	2.30 ± 0.04 (5)	1.00 (10)
Molecular length (kb)	308.7 ± 9.1	217.2 ± 4.0	94.5
Frequency in sample	0.21	0.31	0.48
C. From KF532 (F14(P132))			
Molecular length (F units)	3.31 ± 0.09 (3)	2.30 ± 0.07 (6)	1.00 (6)
Molecualr length (kb)	312.6 ± 8.5	217.8 ± 6.8	94.5
Frequency in sample	0.10	0.25	0.65
D. From KF533 (F14(P132))			
Molecular length (F units)	3.32 ± 0.07 (10)	2.23 ± 0.06 (7)	1.00 (6)
Molecular length (kb)	314.3 ± 7.1	210.6 ± 5.3	94.5
Frequency in sample	0.25	0.25	0.50

[a] Number of molecules measured for data

[b] The size of this class was determined using ColEI as an internal standard. Identified as F by independent heteroduplex with F316 and F*ins*58.8.

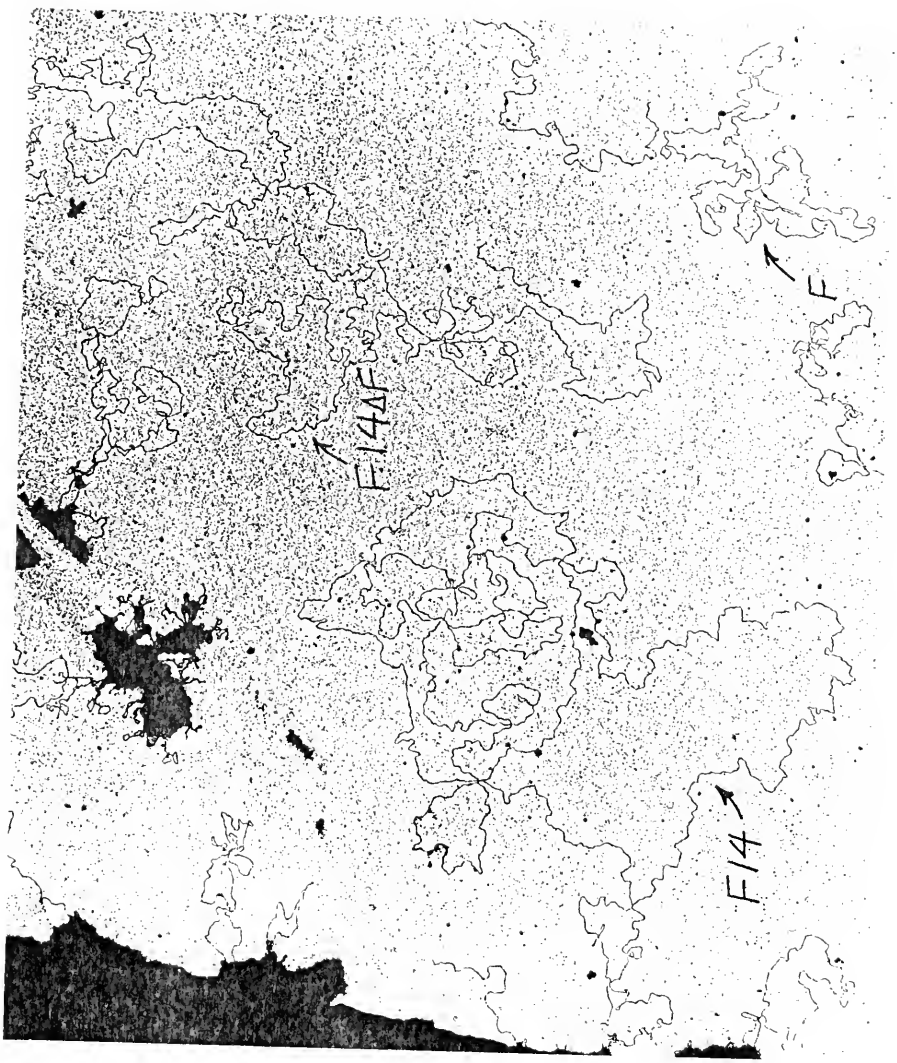
(Hendrickson and Duggan, 1976). It has a delay in transfer of the proximal marker, *met*B, when compared to the transfer of this marker from AB1206. The time-of-entry of *met*B is approximately 17 min, which is similar to the time-of-entry of this marker on the F14 harbored in a *rec*A$^-$ strain (Hendrickson and Duggan, 1976). The most distal marker tested, *ilv*D, comes in five min after *met*B; this is characteristic of the parental F14, whether it is harbored in AB1206 (χ1254), in *rec*A$^+$ diploid or in *rec*A$^-$ diploid strains (Pittard and Adelberg, 1963; Hendrickson and Duggan, 1976).

The plasmid DNA extracted from each of the *rec*A$^-$ strains carrying transduced F14s fall into three size classes (Table 2) (Figure 2). The smallest class of molecules has been shown to be of the same size as F, 94.5 kb. For these measurements ColEI was used as a reference molecule. The other two size classes are either of the F14 class (311 kb) or of the F14ΔF subunit class (216.5 kb). For these measurements the F class molecules were used as reference molecules.

The genetic data (Hendrickson and Duggan, 1976) and the contour length classes of the "F14-like" transductants are similar to those determined for the parental F14 and are indistinguishable from F14.

In the isolations of each of the different transduced F14 plasmid DNA, the frequency of occurrence of each size class differed in the final preparation. This was also true for independent isolations of the same plasmid DNA. The differences in the frequency of occurrence is probably due to slight variations in the isolation procedure and not to the plasmid DNA distribution *in vivo*. The plasmid DNA extracted from F14(P133), as shown in Table 2, gave a

Figure 2. Electron micrograph of size class molecules in the plasmid population of KF533 [F14(P133)]. Note the presence of the 3.3 times (lower left-hand corner), 2.3 times (upper right-hand corner) and 1.0 times F (lower right-hand corner) molecules present in the sample.


F14ΔF
F
F14

higher frequency of the F14 class to F class molecules than any other isolation. It also had the highest concentration of DNA (20 μg/ml). F14(P133) was used as the representative of transduced F14s in all further experiments.

We have shown that both the parental F14 strain (χ1254) and the strains carrying "F14-like" plasmids from transductants contain the same sizes of plasmid DNA. These molecules, even though they are of the same contour lengths, could be of slightly or greatly different base sequences. It seems necessary to show that the molecules of DNA that had been transduced are the same as those in the parental F14 strain by criteria other than that previously discussed. One method for demonstrating sequence identity is heteroduplex analysis.

Heteroduplex Analysis

To determine if the relevant structure of the molecules from the different size classes of transduced F14 derivatives were the same as the structures of the different molecules from size classes of parental F14, several heteroduplex experiments were carried out. Using F*ine*58.8 (Palchaudhuri, Maas and Ohtsubo, 1976), and one of the F*ilv*'s, F316, generated from F14 by P1 transduction (Pittard and Adelberg, 1963; Ramakrishnan and Adelberg, 1965; Marsh and Duggan, 1972; Lee, Ohtsubo, Deonier and Davidson, 1974), as reference molecules in heteroduplex analysis, we proposed to determine the structure of the F class molecules and the structures of the junctions of F DNA and chromosomal DNA on the F14 class molecules. A self-renatured sample, that is, the F14 class molecules heteroduplexed with the F class or the F14ΔF subunit class from the same

plasmid isolation would demonstrate that both of the size classes were subunits of the transduced F14 (Ohtsubo *et al.*, 1974A; Deonier *et al.*, 1974).

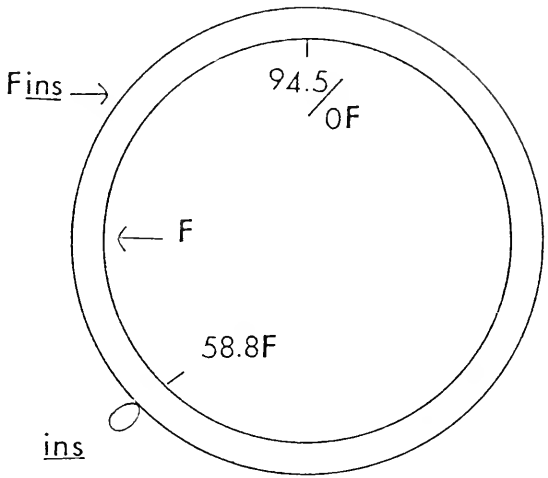
(i) The Reference Molecules

$F_{ins58.8}$ and F316 are well characterized F-primes. They appear in Figure 3 as heteroduplex molecules with F to indicate their structure. $F_{ins58.8}$ has the complete F sequence (94.5 kb) plus an insertion at 58.8 kb (1.3 kb) giving it a molecular length of 95.8 kb (Palchaudhuri *et al.*, 1976). F316 is a deletion mutant of F14 and carries a small insertion (0.8 kb) at 78.6F. It has a deletion in the F14 sequence from 6.9B to 11.4F. This can be depicted in a heteroduplex with F (Figure 3) as a 6.9 kb insertion of chromosomal DNA (OB to 6.9B) and a 2.9 kb deletion of F DNA (8.5 to 11.4F) (Lee *et al.*, 1974). F316 has a molecular length of 99.8 kb.

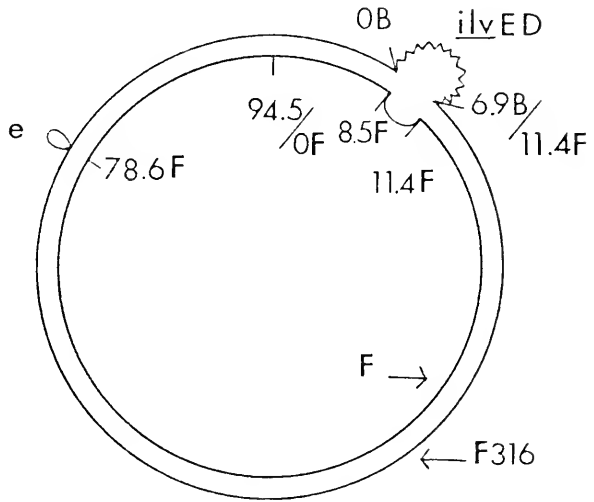
(ii) Self-renatured Heteroduplex Structures

For this experiment a sample of plasmid DNA from KF533[F14(P133)] was denatured and then self-renatured. We had expected, in addition to homoduplexes of the three size classes, heteroduplex structures between the three size classes. However, because of multiple nicked strands in the F14 class and the F14ΔF subunit class, we were unable to find complete homoduplexed molecules or heteroduplexed structures in these size classes. Others who have studied the F14 did not find heteroduplexed structures of these molecules at a high frequency (E. Ohtsubo, personal communication). We did, however, find some complete circular structures with the F class molecules (Figures 4 and 5). The structure consisted of a covalently closed double stranded

Figure 3. Heteroduplex structures of reference molecules (a) $F_{ins}58.8$ and (b) F316 with F (Palchaudhuri *et al.*, 1976; Lee *et al.*, 1974). See text and Figure 1 for explanation of notations and coordinates. The insertion loop "e" in F316 is at 78.6F.


Fins
94.5/0F
F
58.8F
ins

(a)


OB
ilvED
e
94.5/0F
8.5F
6.9B/11.4F
78.6F
11.4F
F
F316

(b)

Figure 4. Electron micrograph of a self-renatured heteroduplex of F[F14(P133)] /F14(P133). Interpretation is depicted in Figure 5. See text for explanation.

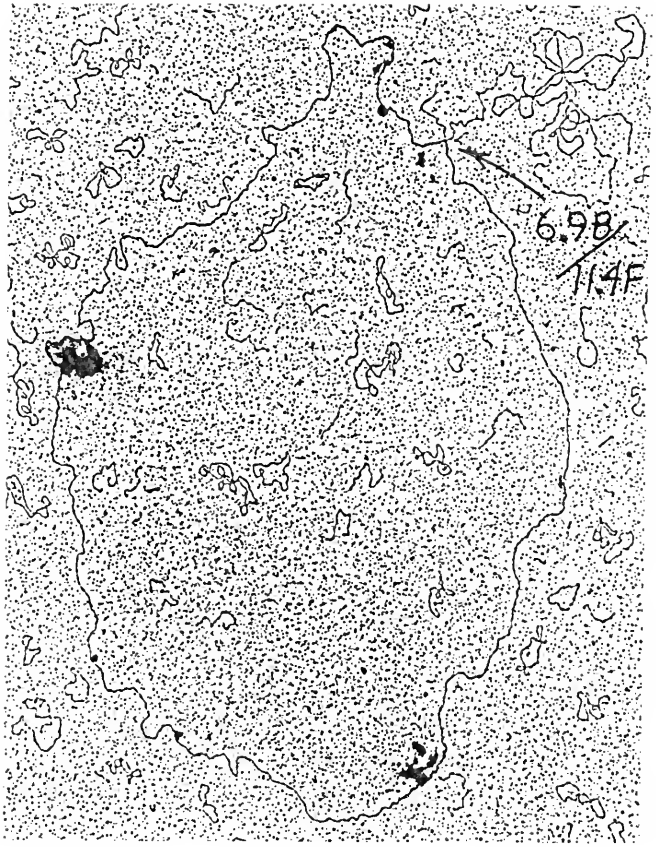
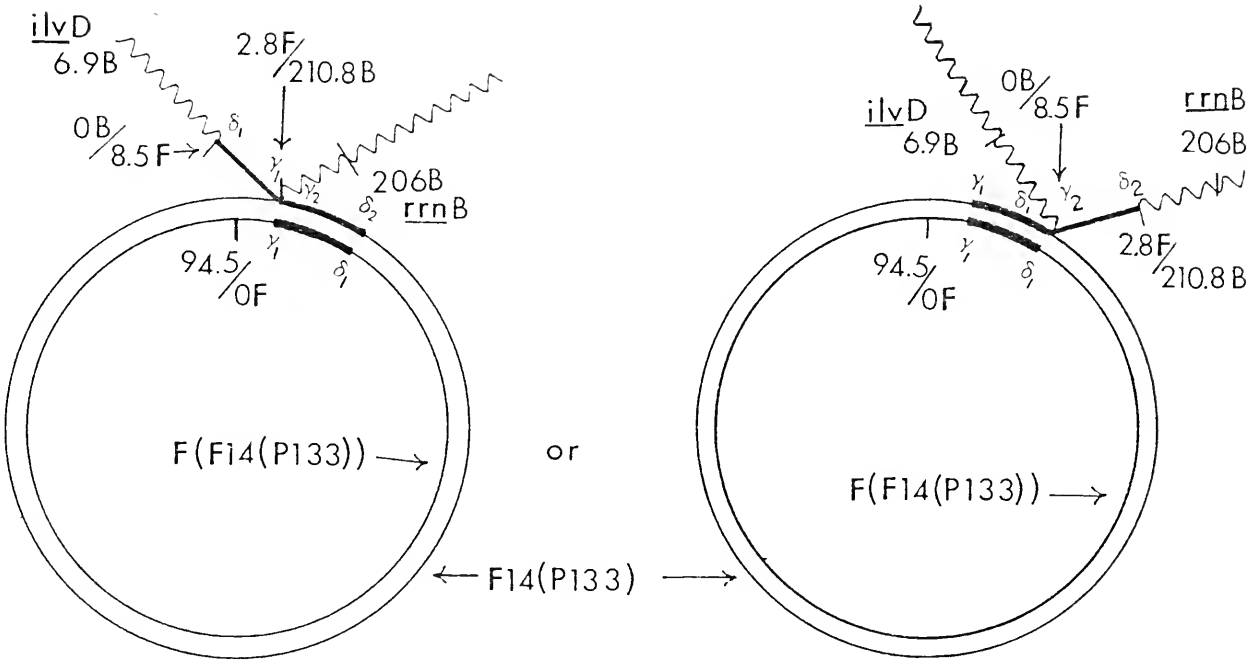

6.98
114F

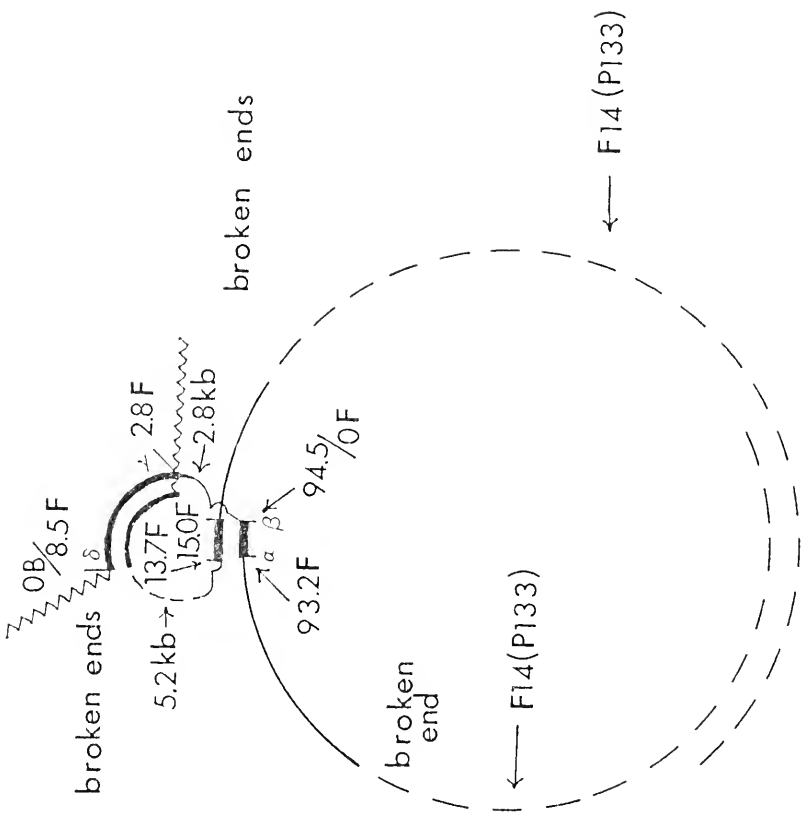
Figure 5. Self-renatured structure: F[F14(P133)]/F14(P133) (F class/F14 class). This figure is an interpretation of Figure 4. The coordinates are based on the structure of F14. This is a completely duplexed circle having a point junction with two ssDNA strands emanating from it, which could occur anywhere between the two structures shown here according to the branch migration hyopthesis (Ohtsubo *et al.*, 1974a). If F14(P133) has the directly repeated sequence γδ (2.8 to 8.5F) at the F DNA-chromosomal DNA junction, then either γδ sequence could completely duplex or partially duplex with the single γδ sequence on the F subunit.


ilvD
6.9B
2.8F/210.8B
0B/8.5F
206B
rrnB
94.5/0F
F(F14(P133))
or
F14(P133)
0B/8.5F
ilvD
6.9B
rrnB
206B
2.8F/210.8B
94.5/0F
F(F14(P133))

DNA (dsDNA) circle plus a point junction from which two single stranded DNAs (ssDNA) emanated. The closed dsDNA circle has a molecular length of 94.5 ± 1.0 kb. The single strands were viable in length and were never seen linked to one another. We propose that this is the F class molecule deplexed to the F segment of a broken F14 class molecule with the remaining single stranded portion missing, as depicted in Figure 5. This structure demonstrates that the F class was part of the structure of the F14 class molecules.

The other heteroduplexes formed were out-of-register structures. This phenomenon occurs when DNA molecules have directly repeated sequences (Ohtsubo *et al.*, 1974a). The F14 has three directly repeated sequences as shown in Figure 1). Ohtsubo *et al.* (1974a) found three heteroduplex structures that demonstrated duplexing between the directly repeated sequences of either αβ or γδ, or both. Among the self-renatured heteroduplex of F14(P133) we found one of these structures as interpreted in Figure 6. We deduced this structure to be a circular structure that has two fixed-length, double-forked duplexes involving the γδ (2.8 to 8.5F) and αβ (93.2 to 94.5F and 13.7 to 15.0F) sequences. There are two single stranded segments completing the circle measuring 2.8 kb (0 to 2.8F) and 5.2 kb (8.5 to 13.7F), respectively. The other single strands emanating from the junction of the duplexes are chromosomal DNA sequences from the 2.8 to 8.5F duplex and F DNA sequences from the αβ duplex. The 5.2 kb (8.5 to 13.7F) segment was broken in the molecule shown here, but the rest of the structure is exactly as described by Ohtsubo *et al.* (1974a). This structure suggests that the αβ sequence

Figure 6. Out-of-register circular structure due to the directly repeated sequences: the $\gamma\delta$ (2.8 to 8.5F) sequence and the $\alpha\beta$ F sequence in a self-renatured heteroduplex of F14(P133). See text for detailed explanation. Broken lines represent the structure if ssDNA strands are broken


broken ends
0B/8.5F
δ
5.2kb
13.7F
15.0F
2.8F
2.8kb
broken ends
α
β
93.2F
94.5/0F
broken end
F14(P133)
F14 (P133)

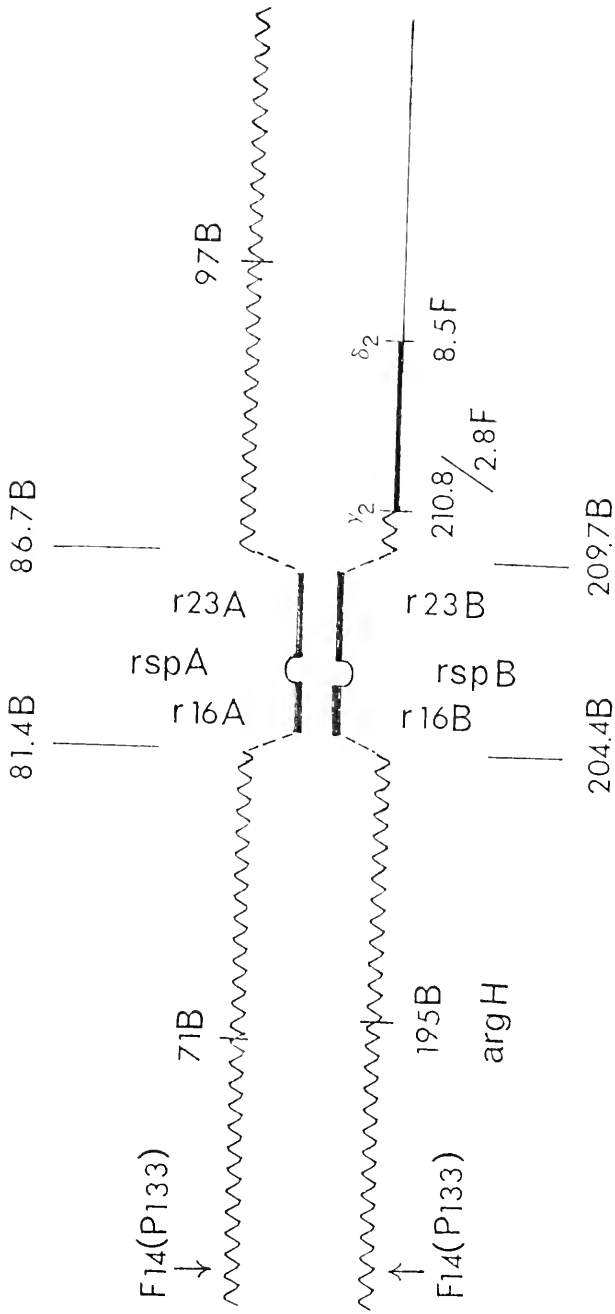
and the γδ (2.8 to 8.5F) are directly repeated in the F14(P133) sequence as it is in the F14.

The other sequences repeated in F14 are the rRNA gene sets. The *r*16 and *r*23 DNA are identical in sequence, but the spacers (*rsp*A and *rsp*B) differ in sequence. If these genes were to duplex, one would see a double-forked duplex, 5.3 kb in length, with a small non-homology loop of 0.3 kb (the spacers) dividing the total duplex into two sub-regions of 1.9 kb and 3.1 kb (*r*16 and *r*23, respectively). We found such an out-of-register structure among the self-renatured structures of F14(P133) (Figure 7). This confirms that the sequence of F14(P133) has both of the rRNA gene sets (*rrn*A and *rrn*B) on its structure as found on F14.

The self-renatured structures confirm three points about the transduced F14s [F14(P133)]: (a) the F class molecules are part of the structure of F14 class molecules, (b) F14(P133) has directly repeated sequences similar to αβ and γδ (2.8 to 8.5F) sequences found on F14 and (c) there is a directly repeated sequence with the characteristics of a heteroduplex between *rrn*A and *rrn*B as found on F14.

We also heteroduplexed F14(P133) with F14. This would be the same as the self-renatured heteroduplex, if the structure of F14 and F14(P133) were the same. We saw no new structures in this heteroduplex that were not found in the self-renaturation.

Figure 7. Out-of-register structure involving the *inmA* and the *inmB* gene sets found in a self-renatured heteroduplex of F14(P133).


F14(P133)
71B
195B
argH
F14(P133)
81.4B
86.7B
rspA
r16A
r23A
97B
δ2
γ2
r16B
r23B
rspB
210.8
2.8F
8.5F
204.4B
209.7B

(iii) $F_{ins58.8}$ Heteroduplex Structures

Two different structures can be predicted when $F_{ins58.8}$ is heteroduplexed with the plasmid molecules of F14(P133). The first structure is identical to a $F_{ins58.8}$/F heteroduplex (Figure 3), that is, a complete duplexed molecule with a 1.3 kb insertion at 58.8F. We found several molecules that had this structure. These results confirm that the F size class molecule in the transduced F14 plasmid population is F. The second structure is a completely duplexed circle with a 1.3 kb insertion (58.8F) with two single strands emanating from a point junction between 38.5 and 44.2 kb due to branch migration (see Figure 5) between the two $\gamma\delta$ (2.8 to 8.5F) sequences. We only found structures with the junction at 2.8F (Figure 8). There were very few complete circles in this preparation. These results confirm that F is a part of the F14(P133) sequence and that the 2.8 to 8.5F sequence may be on the right hand junction of the F DNA/chromosomal DNA junction.

(iv) F316 Heteroduplex Structures

The structure of the heteroduplex F316/F14(P133) is shown in Figures 9 and 10. This was a complete duplex circle with two single strands emerging from a point junction: 30.3 ± 0.5 kb from a 0.8 kb insertion loop. This structure is the same structure one would find in a F316/F14 heteroduplex. This structure confirms that F14(P133) has the 6.9 kb chromosomal sequence that includes *ilv*E and part of *ilv*D, the 2.8 to 8.5F sequence on the left junction and a sequence that is similar to the F sequence of F14.

Figure 8. Heteroduplex structure of F$_{\gamma\gamma s\ 58.8}$/F14(P133). This is a completely duplexed circle with two ssDNA strands emerging from a point junction. If the $\gamma\delta$ (2.8 to 8.5F) sequence were duplicated in F14(P133) as they are in F14, one would find a variety of duplexed circles with the point junction between 2.8F and 8.5F as shown in Figure 5, due to the $\gamma\delta$ branch migration hypothesis. See text for explanation of this heteroduplex. The structure shown here had broken ssDNA strands and therefore was not complete for the chromosomal sequence.

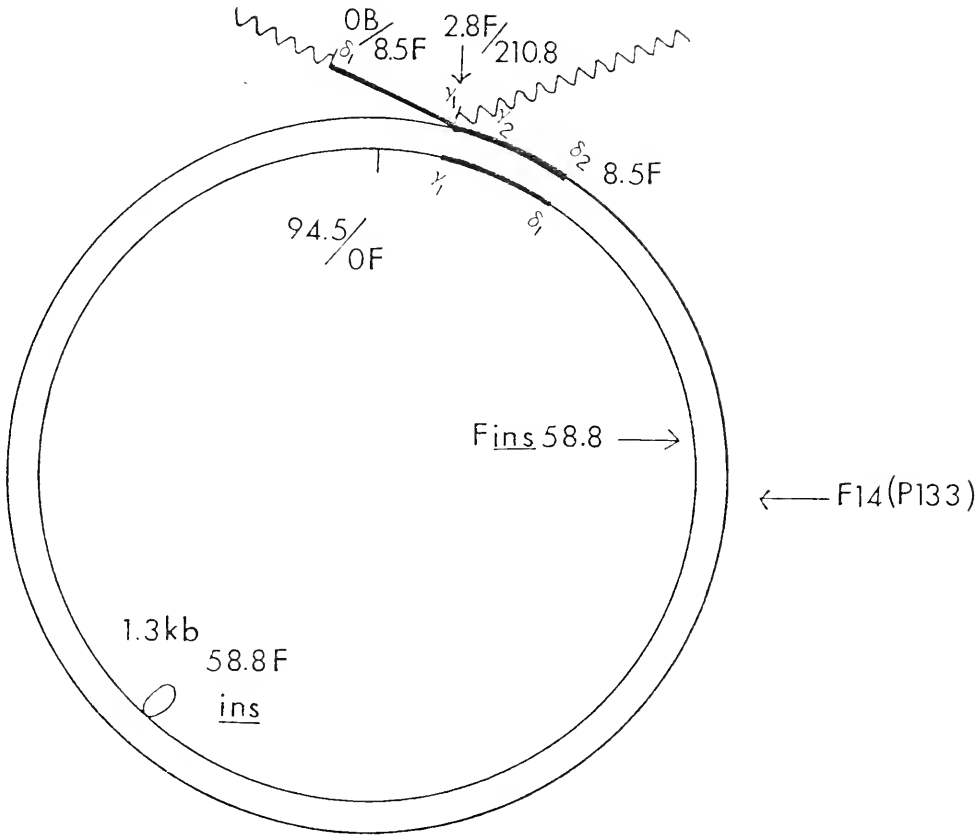

0B
8.5F
2.8F
210.8
δ1
γ1
ε1
ε2
δ2
8.5F
γ1
δ1
94.5
0F
Fins 58.8
F14(P133)
1.3kb
58.8F
ins

Figure 9. Electron micrograph of F316/F14(P133). Interpretation is depicted in Figure 10. See text for explanation.

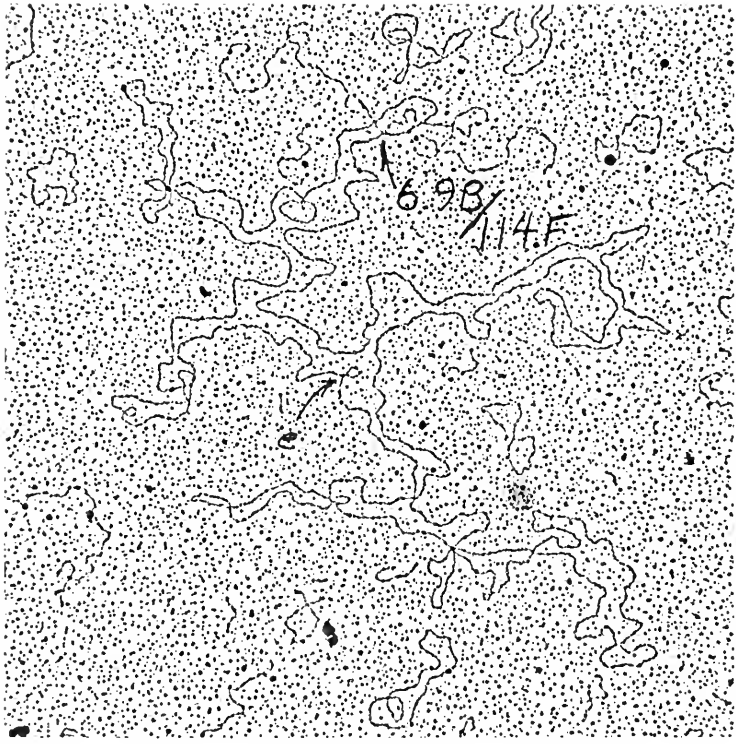
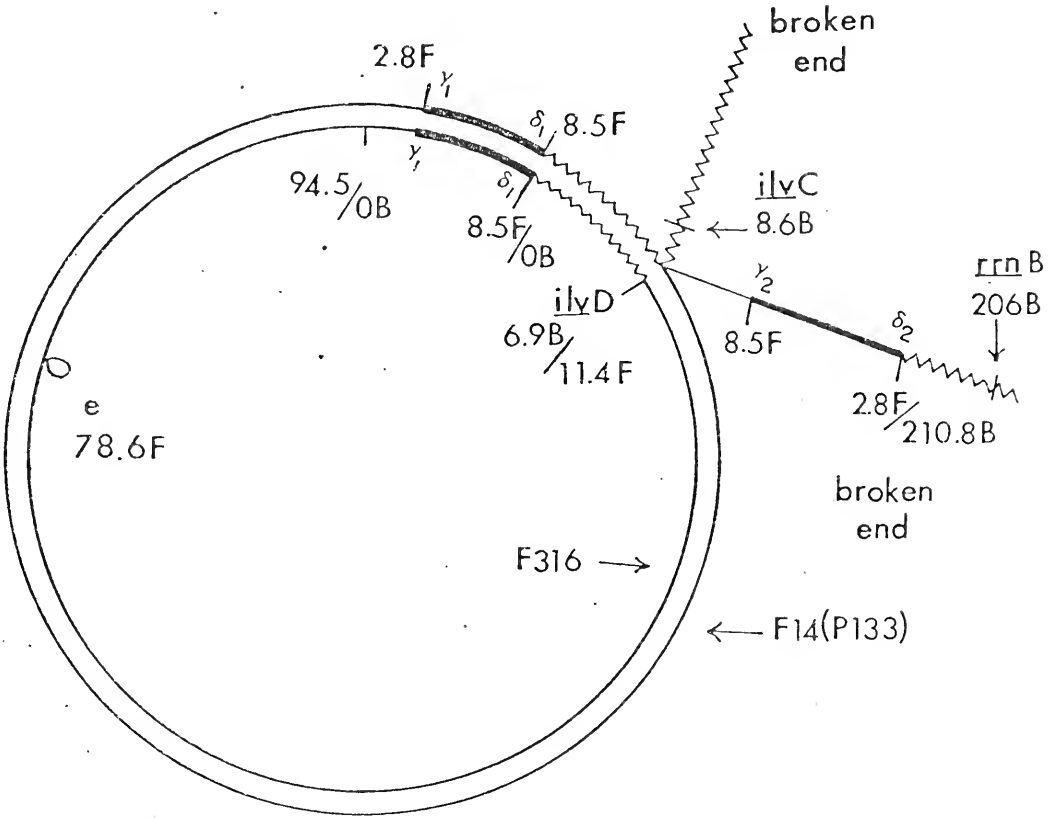

1698/14F

Figure 10. Heteroduplex structure of F316/F14(P133). The insertion (0.8 kb) at 78.6F is identified as "e." The $\gamma\delta$ (2.8 to 8.5F) sequence is identified with bold lines and subscripts ($\gamma_1\delta_1$ is the 2.8 to 8.5F sequence next to the 8.5F/0B junction and $\gamma_2\delta_2$ is the 2.8 to 8.5F sequence next to the 210.8B/2.8F junction).


broken
end
2.8F
γ1
δ1
8.5F
γ1
94.5
0B
δ1
8.5F
0B
ilvC
8.6B
rrn B
206B
γ2
δ2
8.5F
ilvD
6.9B
11.4F
2.8F
210.8B
e
78.6F
broken
end
F316
F14(P133)

Another structure found in this heteroduplex is shown in Figures 11 and 12. This was a linear structure with the 78.6F insertion loop. At a distance of 24.4 kb from the insertion loop there was a substitution loop with two arms having lengths of 6.9 kg and 2.9 kb, respectively. The 6.9 kb arm would correspond to the chromosomal sequence of F316, carrying the *ilv*E gene. The 2.9 kb arm would be the F DNA (8.5 to 11.4F). This structure would be expected in a F316/F heteroduplex, as shown in Figure 3. This suggests once again, that the F sequence on the F14(P133) is similar to F and that the F class molecules may be subunits of F14(P133) as F is a subunit of the F14 (Ohtsubo *et al.*, 1974a).

The structure in Figures 13 and 14 was also found in the F316/F14(P133) heteroduplex. This was a linear duplex molecule with a substitution loop with two arms having lengths of 6.9 kb and 2.9 kb, respectively, followed by a duplex region having a length of 5.7 kb and two single strands emanating from its other end. This 5.7 kb duplexed region is interpreted as an out-of-register structure between F316 and F14(P133), involving the $\gamma_2\delta_2$ (2.8 to 8.5F) sequence on the right-hand junction of F14(P133) and $\gamma_1\delta_1$ (2.8 to 8.5F) of F316. The 6.9 kb substitution arm is the chromosomal DNA segment of F14(P133). The single strands emerging from the other end are the chromosomal sequence of F14(P133) and the F sequence from F316; this single stranded point junction indicates the 210.8B/2.8F junction of F14(P133) in a complete F14/F316 heteroduplex. This structure and the previous structure demonstrate that the F14(P133) has the directly repeated

Figure 11. Electron micrograph of a heteroduplex structure of F316/F14(P133). See Figure 12 and text for explanation.

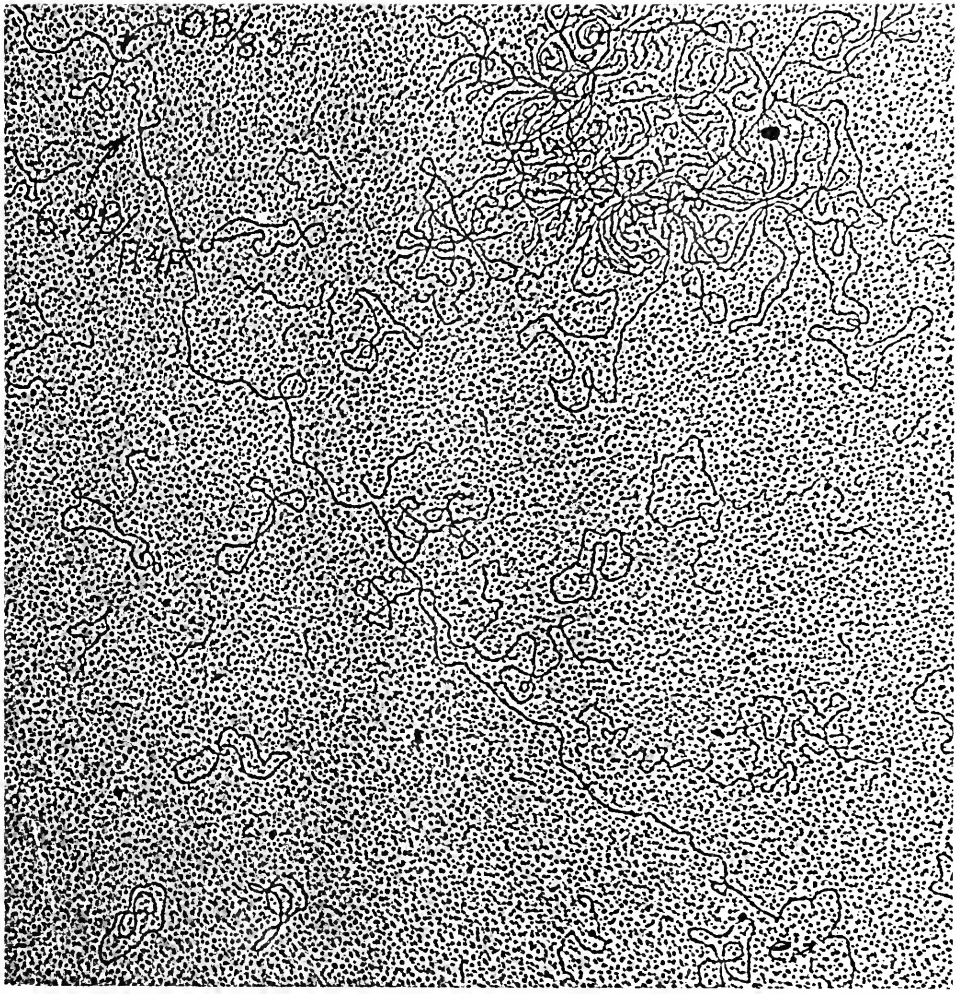


Figure 12. Heteroduplex structure of F316/F14(P133). This structure involves a heteroduplex between broken segments of F316 and the F class subunit of F14(P133). The insertion at 78.6F in F316 is identified by "e." The $\gamma\delta$ (2.8 to 8.5F) sequences are denoted by bold lines.

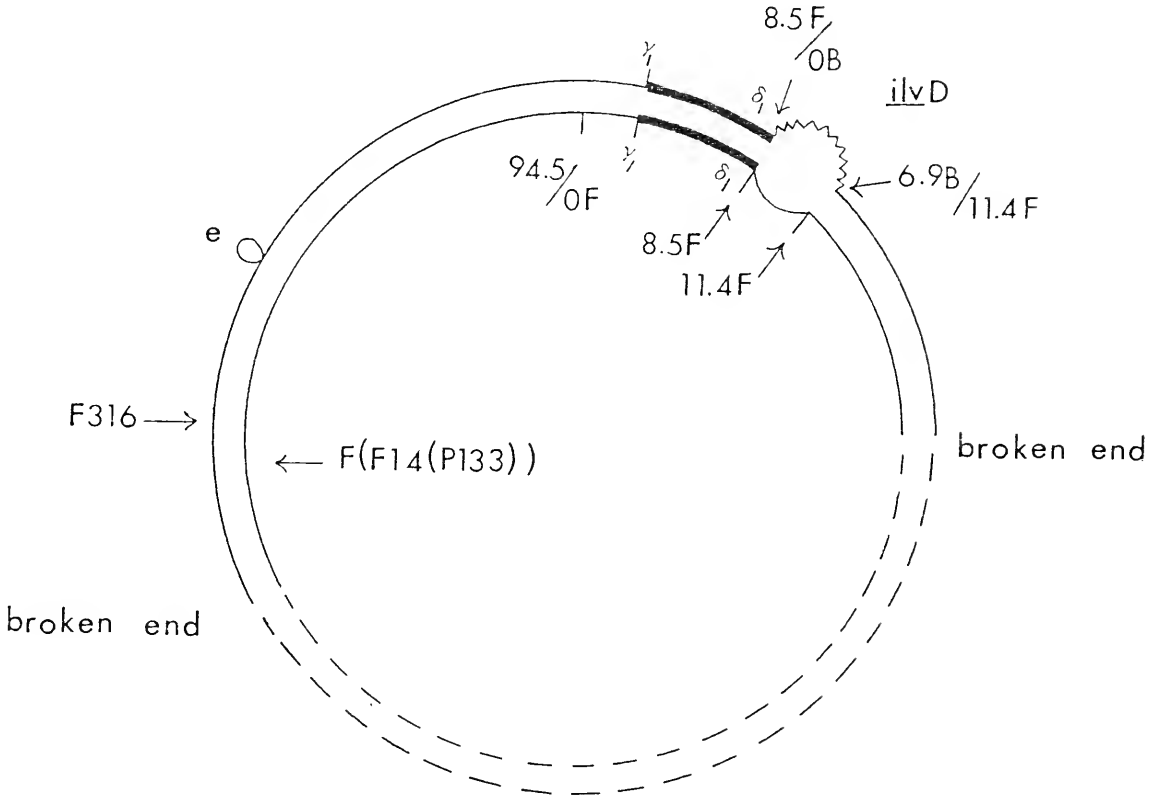

8.5F
0B
ilvD
94.5
0F
6.9B
11.4F
8.5F
11.4F
e
F316
F(F14(P133))
broken end
broken end

Figure 13. Electron micrograph of a heteroduplex structure of F316/F14(P133). This is an out-of-register structure. See Figure 14 and text for explanation.

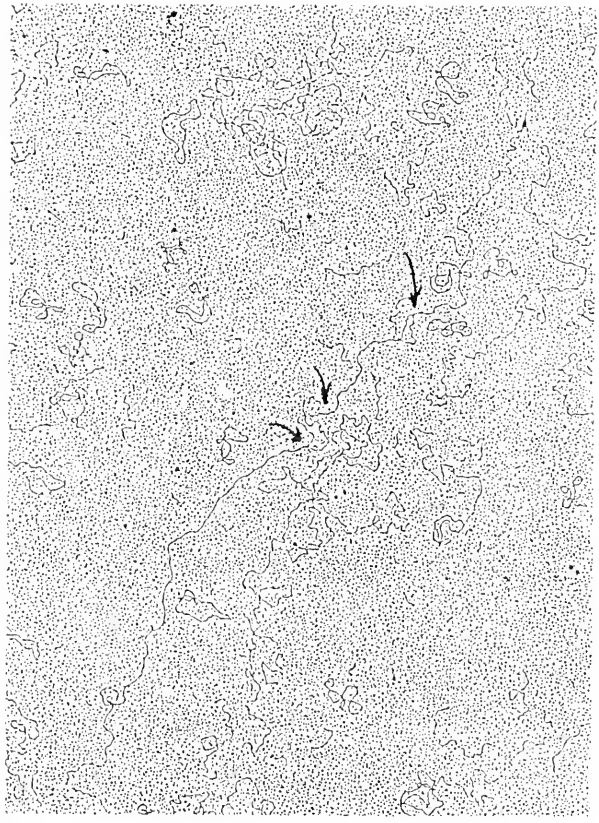
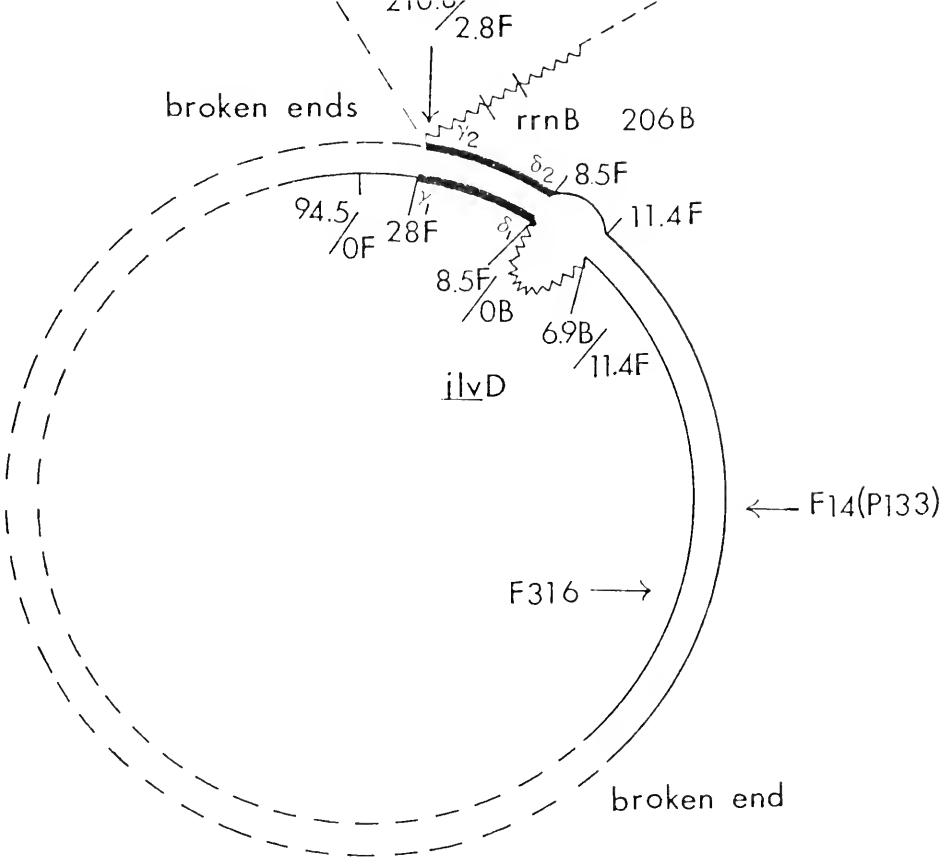


Figure 14. Heteroduplex structure of F316/F14(P133). This is an out-of-register duplex between the $\gamma\delta$ (2.8 to 8.5F) sequence on F316 and $\gamma_2\delta_2$ (2.8 to 8.5F) sequence on F14(P133). The substitution loop is the δ_2 to 6.9B sequence of F316 and the 8.5 to 11.4F sequence of F14(P133). The $\alpha_1\beta_1$ sequence is shown at 13.7 to 15.0F.


2.8F
broken ends
rrnB 206B
γ_2
δ_2
8.5F
11.4F
94.5
0F
28F
γ_1
δ_1
8.5F
0B
6.9B
11.4F
ilvD
F14(P133)
F316
broken end

sequence of 2.8 to 8.5F on both of its F DNA and chromosomal DNA junctions, identical to the F14 (Ohtsubo *et al.*, 1974a).

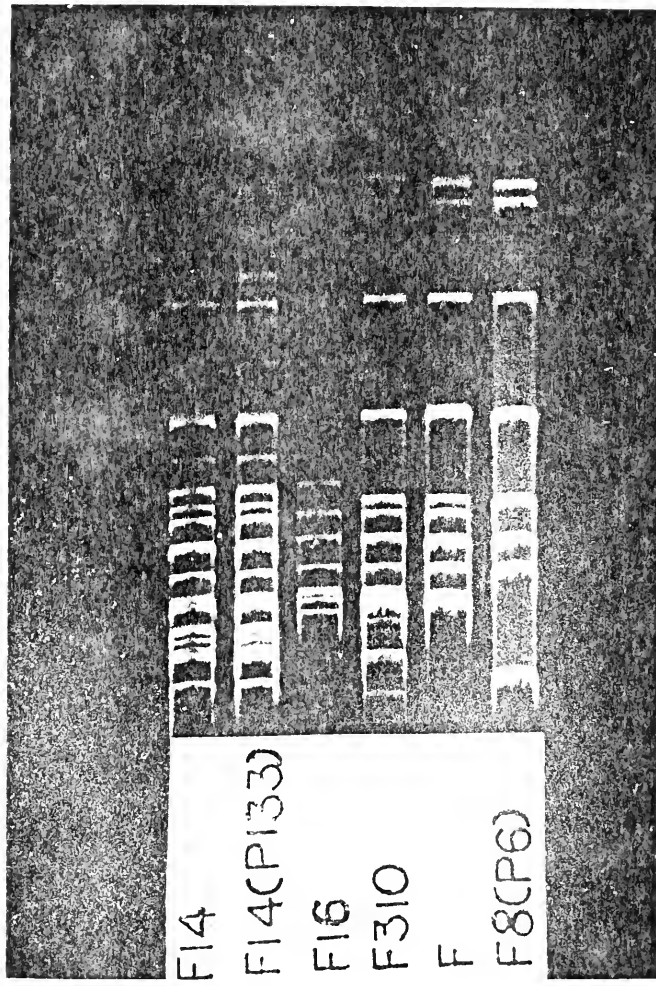
To this point we have established three general facts about the transduced F14 structure. Firstly, there are three size classes found among plasmid DNA isolated from *rec*A$^-$ strains carrying these plasmids; each size is identical to those of the F14, 3.3, 2.3 and 1.0 times the size of F (Ohtsubo *et al.*, 1974a). Secondly, the F class molecules are part of the transduced F14's structure. And thirdly, the F DNA/chromosomal DNA junctions have directly repeated $\gamma\delta$ (2.8 to 8.5F) sequences, identical to the structure of F14. We have not been able to show the relationship of the remaining structure of the F14 class molecules to the F14 because we did not have any other reference DNA molecules carrying sequences of the F14 chromosomal segment.

Gel Electrophoresis of *Eco*R1 Fragments

It has been demonstrated that certain restriction endonucleases (such as *Eco*R1) will make double stranded breaks at specific sequences (Hedgepeth, Goodman and Boyer, 1972). They will cleave homologous DNA into specific and reproducible fragments. Gel electrophoresis has been used on an analytical scale to separate these fragments into bands based on molecular weight (length) (Sharpe, Snyder and Sambrook, 1973).

*Eco*R1 cleavage of F14 and related plasmids F, F310 and F16 could be used to identify the *Eco*R1 fragments of a transduced F14 by gel electrophoresis. Figure 15 shows typical *Eco*R1 fragments in an agarose gel. The restriction fragment pattern of F14(P133) is

Figure 15. Agarose gel (0.5%) electrophoresis of the *Eco*R1 cleavage products of DNAs from (left to right) (a) F8(P6), (b) F, (c) F310, (d) F16, (e) F14(P133) and (f) F14. Explanation and interpretation of bands are in Figure 16.


F14
F14(P133)
F16
F310
F
F8(P6)

identical to that of F14. Figure 16 and Table 3 summarize the data obtained in 0.7% and 0.5% agarose gels, and 4.0% polysacrylamide gels. We have identified 44 bands shared by both F14(P133) and F14. They range in size from 0.38 kb to 26 kb and account for 297.5 kb of 311 kb of F14. We can identify all 18 fragments of F greater than 0.38 kb (E. Ohtsubo and H. Ohtsubo, personal communication; Skurry, Nagaish and Clark, 1976; Childs, H. Ohtsubo, E. Ohtsubo, Sonnenberg and Freundlich, 1977), demonstrating, once again, that the F size class molecules are F.

Childs *et al.* (1977) have mapped *Eco*R1 fragments of F310 and F312 which are F14 deletion mutants, carrying chromosomal sequences in the *ilv* region. Both carry a large fragment, x24 (11.9 kb), which has a restriction site at 4.7F and 8.1B (F14 coordinates). This fragment contains the 8.5F/OB junction and all the chromosomal DNA through the *ilv*A gene. Our results show that F16, F14 and F14(P133) have the same x24 fragment, further evidence of the structure of this junction in transduced F14s.

The restriction map of the *ilv* region also shows four other fragments: x27, x28, x29 and x30 (1.25 kb, 1.05 kb, 0.87 kb and 0.63 kb, respectively) (Figure 17) (Childs *et al.*, 1977). These fragments account for the sequence from the *ilv* region to 12.2B, F16, F14 and F14(P133) carry all of these fragments.

F16 has two additional fragments as seen in Figure 16, x32 amd x33 (13.8 kb and 6.9 kb, respectively). The x33 fragment is also shared with F14 and F14(P133). This fragment must, therefore, be the next segment in the clockwise direction from the *ilv* region. The chromosomal region through 19.1B can now be identified on F14 and F14(P133).

Figure 16. *Eco*R1 generated fragments of F8(P6), F, F310, F16, F14(P133) and F14 greater than 0.38 kb. These patterns were derived from electrophoresis in 0.5% and 0.7% agarose gels, and in 4% polyacrylamide gels. F fragments are denoted by broken lines and in decreasing order or molecular weight (MW) by f1-f18 (E. Ohtsubo and H. Ohtsubo, personal communication; Childs *et al.*, 1977). Chromosomal fragments are denoted by solid lines and numbers preceded by x. Fragments x24 and x27-x30 denote chromosomal DNA in *Filv*'s in decreasing order of MW (Childs *et al.*, 1977). Fragments x32 and x33 denote two new *Filv* chromosomal fragments found on F16 in decreasing MW. F14 and F14(P133) share fragments x24, x33, and x27-x30 with *Filv*'s. The remaining chromosomal fragments are denoted by x34-x53 in decreasing order of MW. F8(P6) fragments were the internal standards used in all gels. They are denoted by solid lines and are numbered 1-10 in decreasing order of MW.

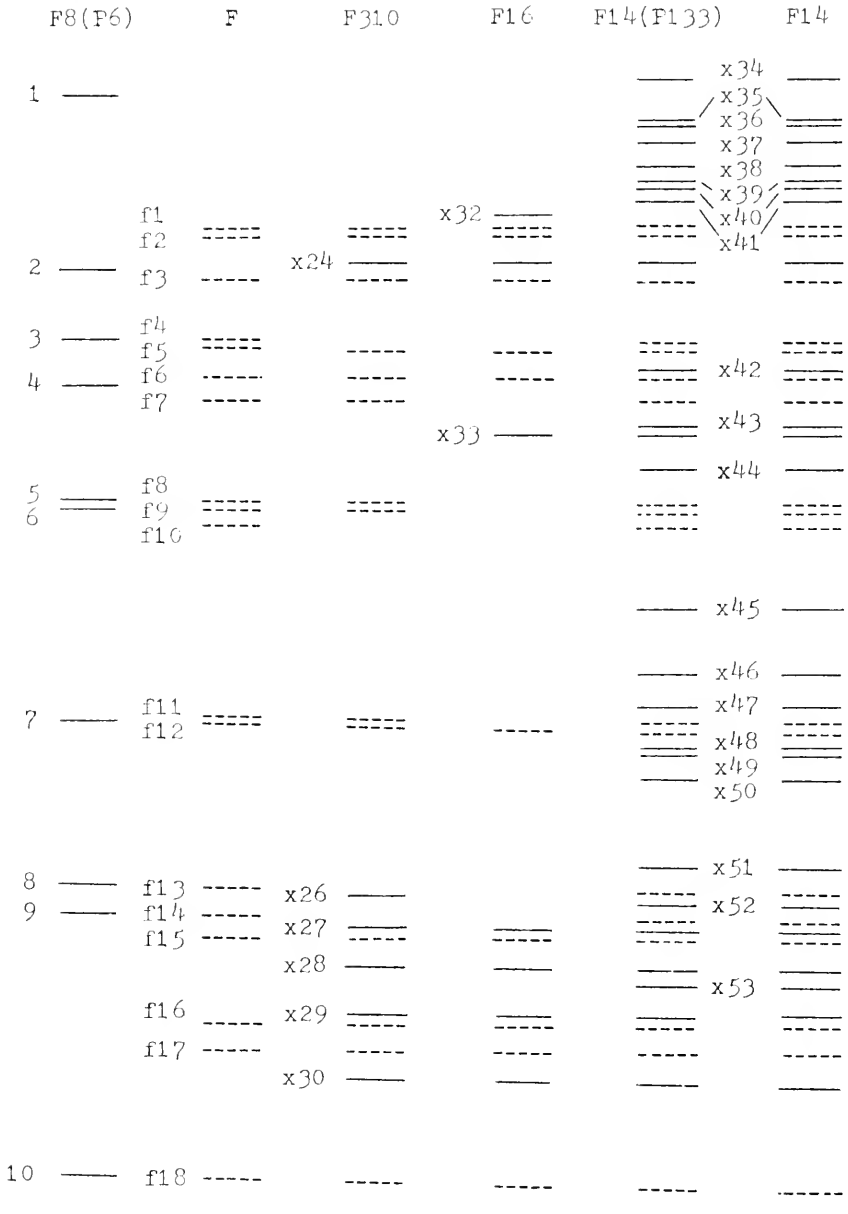
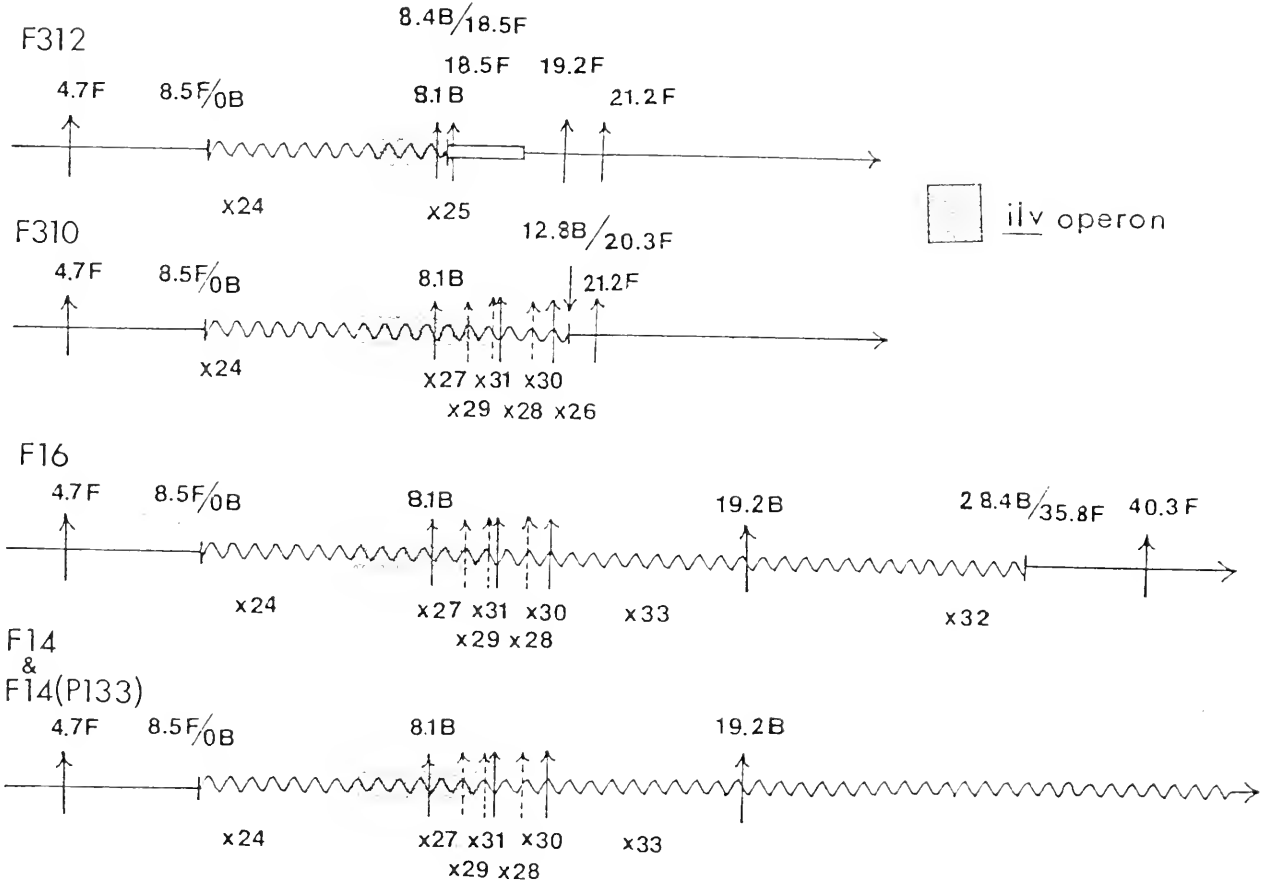

F8(P6)
F
F310
F16
F14(F133)
F14
1
2
3
4
5
6
7
8
9
10
f1
f2
f3
f4
f5
f6
f7
f8
f9
f10
f11
f12
f13
f14
f15
f16
f17
f18
x24
x26
x27
x28
x29
x30
x32
x33
x34
x35
x36
x37
x38
x39
x40
x41
x42
x43
x44
x45
x46
x47
x48
x49
x50
x51
x52
x53

Table 3. *Eco*R1 fragments of DNAs from F14(P133), F14, F16 and F310

Chromosomal DNA fragments					F DNA fragments				
Fragment	MW[a]	F14 or F14(P133)	F16	F310	fragment	MW	F14 or F14(P133)	F16	F310
x24	11.9	x[b]	x	x	f1	13.5	x	x	x
x26	1.35	-	-	x	f2	13.3	x	x	x
x27	1.25	x	x	x	f3	10.8	x	x	x
x28	1.05	x	x	x	f4	9.2	x	-	-
x29	0.87	x	x	x	f5	9.0	x	x	x
x30	0.63	x	x	x	f6	7.7	x	x	x
x32	13.8	-	x	-	f7	7.3	x	-	x
x33	6.9	x			f8	4.6	x	-	x
x34	26.0	x			f9	4.4	x	-	x
x35	18.0	x			f10	4.3	x	-	-
x36	17.7	x			f11	2.34	x	-	x
x37	17.3	x			f12	2.28	x	x	x
x38	16.0	x			f13	1.40	x	-	-
x39	14.9	x			f14	1.30	x	-	-
x40	14.6	x			f15	1.20	x	x	x
x41	14.2	x			f16	0.75	2x	x	x
x42	8.1	x			f17	0.66	x	x	x
x43	7.1	x			f18	0.38	x	-	x
x44	6.5	x							
x45	3.7	x							
x46	2.7	x							
x47	2.5	x							
x48	2.2	x							
x49	2.1	x							
x50	2.0	x							
x51	1.5	x							
x52	1.35	x							
x53	1.0	x							

[a] MW determination is explained in Material and Methods (MW in kb units).
[b] Presence of fragment in plasmid DNA sequence represented by (x); absence shown by (-).

Figure 17. *Eco*R1 maps of bacterial DNA in the *ilv* region and surrounding regions of F DNA on F14(P133), F14, F16, F310 and F312. Sawtooth lines denote *E. coli* DNA and solid lines denote F DNA. The open box in F312 is a gap which is included to line up the sequences. The coordinates are placed according to Marsh and Duggan (1972), Lee *et al.* (1974) and Childs *et al.* (1977). Arrows represent the sites recognized by *Eco*R1. Dotted arrows indicate that the order of the fragments is not known. Coordinates of the cutting sites are derived from *Eco*R1 sites on F (E. Ohtsubo, personal communication; Skurry *et al.*, 1976) and molecular lengths of the bacterial fragments (Childs *et al.*, 1977).


F312
8.4B/18.5F
18.5F
19.2F
4.7F
8.5F/0B
8.1B
21.2F
x24
x25
ilv operon
F310
12.8B/20.3F
4.7F
8.5F/0B
8.1B
21.2F
x24
x27 x31 x30
x29 x28 x26
F16
4.7F
8.5F/0B
8.1B
19.2B
28.4B/35.8F
40.3F
x24
x27 x31 x30
x29 x28
x33
x32
F14
&
F14(P133)
4.7F
8.5F/0B
8.1B
19.2B
x24
x27 x31 x30
x29 x28
x33

The x32 fragment of F16 accounts for the remainder of the chromosomal region through the 28.4B/35.8F junction to 40.3F, which is the *Eco*R1 restriction site between fragments f7 and f5 on the *Eco*R1 map of F (E. Ohtsubo and H. Ohtsubo, personal communication). For obvious reasons, F14 and F14(P133) do not carry this fragment. The chromosomal region fragments x37, x46, x49 and x51, shared by F14 and F14(P133), can also be tentatively identified by molecular lengths. The *ppc-arg*ECBH-*bfe* region, which is found on the F14, have four *Eco*R1 fragments (Devine, Moran, Jederlinic, Mazaitis and Vogel, 1977). The F14 *Eco*R1 fragments that correspond to these chromosomal fragments can be arranged as they appear clockwise on the chromosomal map as follows: x49, x37, x46 and x51 (2.1 kb, 17.3 kb, 2.7 kb and 1.5 kb, respectively). The x37 fragment carries the *ppc* gene and *arg*ECBH region. The x51 fragment carries *bfe* (Devine *et al.*,1977). The *Eco*R1 site, left of the *bfe* gene, is probably in or near the *rrn*B gene set.

Since *Eco*R1 sites occur in the γδ (2.8 to 8.5F) sequence, one should not see any new fragments generated from the F14ΔF subunit size class. This subunit shares identical *Eco*R1 cleavage sites near the 8.5F/0B and 210.8B/2.8F γδ-chromosomal junctions with F14, the 4.7F site and the 4.0F site, respectively (Skurry *et al.*, 1976; Childs *et al.*, 1977). The 4.7F site attributes to the formation of the x24 fragment of F14 which should be found on the F14ΔF subunit. The 4.0F site generates the f16 fragment of F and the fragment counterclockwise into the chromosomal DNA —— the *rrn*B region; both fragments should be found on the F14ΔB subunit. There are no other *Eco*R1 sites in γδ because the next F fragment counterclockwise from f16 is f12,

2.3 kb in length (*Eco*R1 site is at 1.7F). The f16 fragment is also the only *Eco*R1 fragment that is repeated twice in the F14 structure. F14 and F14(P133) had identical restriction fragment gel patterns in all of the gels run. Any fragments smaller than 0.38 kb could not be determined. This included f19 of F. These results indicate that sequences of F14 and F14s transduced by P1 are indistinguishable. There appears to be no detectable DNA sequences deleted from F14(P133).

Discussion

The transduction of an F-merogenote with properties similar to F14, using P1 lysates made on AB1206(χ1254), the F14 haploid strain, was suggested by Pittard and Adelberg (Bacteriol. Proc., p. 138, 1963) and later supporting evidence was reported by Hendrickson and Duggan (1976). The transduction recipient strains could transfer IlvD$^+$, MetB$^+$ and ArgH$^+$ to *rec*A$^-$ strains. In addition, they could transfer other F14 markers (*ilv*EAC, *met*E, and *rha*). It is conceivable that the transduction could have occurred with extensive deletions in the F-merogenote in order to be packaged by P1, but, the genetic evidence does not support this. Transfer kinetic analysis showed that all of the transduced F-merogenotes examined were indistinguishable from the parental F14 in the order of transfer and the genetic distance between the proximal (*arg*H, *met*B) and distal (*ilv*D) markers. These results suggest that the entire F14 or at least a major protion of the F14 may have been transduced.

The physical and molecular data presented in this report support the earlier genetic evidence that "F14-like" merogenotes are indistinguishable from the parental F14 by three criteria. 1) Each of the three "F14-like" merogenotes examined had three size classes of plasmids, 3.3, 2.3 and 1.0 times F, as did the parental F14. 2) Heteroduplex analysis revealed that (a) the 1.0 times F size molecules in "F14-like" DNA extracts are F, (b) the F sequence is part of the F14 size class molecule, (c) the "F14-like" merogenotes have three directly repeated

sequences: the γδ (2.8 to 8.5F) sequence, the αβ set of F, and the rRNA DNA sequences (*r*16 and *r*23) with heterologous spacers of the rRNA gene sets (*rrn*A and *rrn*B), (d) the structures of the F DNA-chromosomal DNA junctions on the transduced "F14-like" merogenotes have the γδ (2.8 to 8.5F) sequence present on the F DNA side of each junction, and (e) the left hand junction (8.5F/OB) has the first 6.9 kb sequence that contains *ilv*E, *ilv*G and part of the *ilv*D sequence found on F316. All of these properties and structures were found to be unique characteristics of the F14 (Ohtsubo *et al.*, 1974a). 3) The gel patterns of *Eco*R1 fragments, generated from an "F14-like" merogenote, F14(P133), demonstrated that the DNA sequence of F14(P133) is indistinguishable from that of parental F14; the two plasmids yielded identical gel fragment patterns. In all, we could account for 297.5 kb of the 311 kb sequence of F14. We could identify F *Eco*R1 fragments, f1 through f18, demonstrating F to be a subunit of both plasmids. We also found that F14 and F14(P133) shared identical fragments with two of the F*ilv*$^+$ deletion mutants of F14, F16 and F310. These included the *ilv* region fragments, x27, x28, x29 and x30, and the x24 fragment. The latter encompassed the 8.5F/OB junction. They also shared the x33 fragment with F16, a 6.9 kb fragment clockwise from the *ilv* region. Other fragments of the F14 chromosomal sequence were identified by thier size when compared to *Eco*R1 fragments of the *ppa-arg*ECBH-*bfe* region, which were determined by Devine *et al.* (1977). These fragments were x37, x46, x49 and x51.

The physical evidence presented here and the genetic evidence reported earlier (Hendrickson and Duggan, 1976) demonstrate that the transduced "F14-like" merogenotes are indistinguishable from the F14. These data do not say how the F14 is packaged and transduced by P1 when

lysates were grown on AB1206(χ1254). They only show that F14s were found in recipients (previously F^-) after transductions using the P1·AB1206(χ1254) lysates. Some preliminary evidence for the mechanism of transduction is reported in Part II.

PART II

BEGINNING OF AN ANALYSIS OF THE MECHANISM OF P1 TRANSDUCTION OF A LARGE F-PRIME, F14

Introduction

Pittard, using a P1 lysate prepared on AB1206, an *Escherichia coli* K12 strain that harbors the F14 and is haploid for the chromosomal region carried on it, reported that he transduced an F-prime that would conjugally transfer proximal and distal markers of the F14 (Pittard and Adelberg, Bacteriol. Proc., p. 138, 1963). Transductants from such matings were characterized and the F-primes generated were shown to be genetically indistinguishable from the F14 (Hendrickson and Duggan, 1976). In the accompanying paper (Part I) we have shown evidence that these "F14-like" plasmids are physically and molecularly indistinguishable from the F14.

The intriguing question to be asked is how did P1 transduce this plasmid that is at least three times larger (205 x 10^6 daltons) than the DNA found in the transducing particles (64 x 10^6 daltons). Ohtsubo (1971) also reported the transduction of F8 by P1, which is larger (77.4 x 10^6 daltons) than the P1 genome. Neither of the transductions would conform to the "headful" hypothesis for packaging DNA proposed by Streisinger, Emrich and Stahl (1967) if the P1 particle used in these transductions were the same size as the normal size phage capsid (1.472 g cm^{-3} or 86 nm head). As

previously reported, the genetic transfer of F14 with P1 lysates were carried out in the presence of nalidixic acid and deoxyribonuclease (DNase) to rule out conjugation and transformation, respectively. Neither of these had any effect on the efficiency of genetic transfer (Hendrickson and Duggan, 1976).

One further suggestion has been that P1 phage particles may play a role in the transformation of these plasmid DNA, acting as phage "helpers," as shown by Kaiser and Hogness (1960) using phage lambda. The calcium concentration used in the transduction procedure is near the lower limits of that used in transformation (Cohen, Chang and Hsu, 1972). In this paper we present further evidence that the mode of genetic transfer is transduction. Other objectives are to determine the number of particles involved in the transduction and the influence of calcium on the transduction, and to find and characterize the phage particles involved.

Materials and Methods

Media

All strains and phage lysates were grown on the media described previously (Hendrickson and Duggan, 1976 and Part I). The selective and minimal media used in the selection of recombinants were the same as described previously.

Bacterial Strains

The bacterial strains used are shown in Table 1.

Bacteriophage

The bacteriophage used was the generalized transducing phage P1*kc* (Lennox, 1955) obtained from Roy Curtiss III (University of Alabama in Birmingham).

Production of Phage Lysates

The methods used for the production of P1 lysates were described previously (Marsh and Duggan, 1972; Hendrickson and Duggan, 1976).

Transduction Procedures

The recipient strains were grown to a concentration of 2×10^9 cells/ml in Z broth containing 2.8×10^{-3} M $CaCl_2$. Transductions were performed at a multiplicity of exposure (m.o.e.) of 1.0, incubated for 20 min at 37°C, chilled, centrifuged, resuspended in 56/2 buffer and plated on selective media. When we looked for the co-transduction of F14 markers *ilv*D$^+$, *met*B$^+$ and *arg*H$^+$, selection was

Table 1. *Escherichia coli* K12 strains

Strain	Sex	Genotype	Source or Derivation
χ1254	F14	(F*ilv*$^+$, *met*E$^+$, *rha*$^+$, *met*B$^+$, *arg*H$^+$)/Δ(min 83 to 88) *thi*-1, *his*-1, *pro*A2, *lac*Y1, *tfr*-3, *str*	Eiichi Ohtsubo State University of New York at Stony Brook
AB1450	F$^-$	*ilv*D16, *arg*H1, *met*B1, *his*-1, *str*, *tsx*[a]	Barbara Bachmann Yale University
AB1472	F$^-$	*ilv*D16, *arg*H1, *met*B1, *str*[b]	Barbara Bachmann Yale University
C600	F$^-$	*leu*, *thr*, *str*, λ$^-$	Phil Harriman Duke University
KF2201	F$^-$	*thi*-1, *met*E46, *trp*-3, *his*-4, *str*, *rha*	This laboratory
KF104	F$^-$	As AB1472, *thy*A1, *rec*A1	This laboratory
KF109	F$^-$	As AB1472, *pur*D13, *rif*	This laboratory
KF117	F$^-$	As KF104, *spc*	This laboratory
KLF15/ JC1553	F115	(F*his*$^+$)/*his*, *met*B, *leu*, *arg*G, *str*, *rec*A1[c]	Anthony Pfister University of Florida
MA3050	F42	(F*lac*$^+$)/*lac*$^-$, *his*$^-$, *pro*$^-$, *rec*A$^-$	Anthony Pfister

[a] This strain also carries *thi*-1, *xyl*-7, *mal*A1, *lac*Y1, *gal*-6, *ton*A1, λ^r, λ$^-$.
[b] This strain also carries *thi*-1, *mal*A1, *lac*Y1, *gal*6, λ^r, λ$^-$.
[c] This strain also carries *mal*, *xyl*, *lac*, *mtl*-2, *gal*, *ton*, *tsx*, λ^r, λ$^-$, *sup*E.
[d] This strain also carries *spc*, *thi*-1, *xyl*, *mtl*, *gal*, *tsx*.

made for all three markers at once. The presence of F14 among transductants was tested by cross-streak complementation with *ilv*D$^+$, *met*B$^+$, *arg*H$^+$ cotransductants against an F$^-$ that was *ilv*D$^-$, *met*B$^-$, *arg*H$^-$ and *rec*A$^-$.

Transformation

Two procedures were used. The first was the same as the transduction procedure described above. The other was the procedure of Cohen *et al.* (1972). The cells were grown in minimal medium to a concentration of 1 x 10^9 cells/ml. The $CaCl_2$ concentration used was 0.03 M. The DNA concentrations were 20 µg/ml.

DNA Isolation for Transformation

The procedure used was modified from Cohen and Miller (1969). Cultures were grown to a final concentration of 2 x 10^9 cells/ml in L broth or 56/2 minimal medium. Cells were washed twice in Tris-HCl buffer (0.05 M, pH 8.0). Cells were resuspended in 25% sucrose, 0.05 M Tris-HCl, 0.075 M EDTA, pH 8.0. Lysozyme was added to a final concentration of 1.0 mg/ml, and the mixture was incubated for ten min at 37^{o}C. Spheroplasts were treated with 1.3 ml of 5% Brij-56 and RNase was added (50 µg/ml). This mixture was incubated at 60^{o}C for 60 min. Sodium lauryl sulfate (0.2%) was added and the mixture was incubated for five min at 25^{o}C to achieve a complete lysis. The lysates were mixed with equal volume of phenol (equilibrated with 25% sucrose buffer), containing 0.08% hydroxyquinoline. This was repeated three times. The final DNA solutions, 150 µg/ml, were dialyzed six times against 0.02 M Tris-HCl, 0.001 M EDTA, 0.02 M NaCl, pH 8.0.

Density Centrifugation of Phage Particles

Approximately 1 x 10^{10} p.f.u. were suspended in 4.5 ml of CsCl solution buffered with 0.01 M Tris (pH 7.5) at a final density of 1.473 g cm^{-3}. This mixture was centrifuged in a Beckman SW39L rotor for 36 hr at 23 krev/min at 15°C. Ten drop fractions from the gradients were collected in 0.3 ml of Z broth. Activity of p.f.u. was determined by appropriate dilutions of the CsCl - Z broth fractions, using KF2201 as an indicator strain. Distribution of transducing capability was determined by adding 0.2 ml of the test strain (2 x 10^9 cells/ml) to one-tenth ml of each CsCl fraction. This mixture was incubated at 37°C for 30 min. One-tenth ml of the mixture was added to three ml of SA-1, plated on selective medium, incubated for 48 hr at 37°C and tested for appropriate markers and/or F14-mediated transfer.

Procedure Showing the Effect of Ca^{++} on the Transduction of F14

The transduction procedure was the same as previously described except for chelation and restoration of Ca^{++} to lysates. The lysates were treated with EDTA (2.8 x 10^{-3} M) to chelate the Ca^{++}. These treatments were left for three hr, for 48 hr and for three weeks prior to their use to make sure EDTA had no detrimental effect on the phage particle. The Ca^{++} concentration was restored to 2.8 x 10^{-3} M when the lysates were mixed with the cells for transduction.

Results

Mode of Genetic Transfer

We had previously ruled out conjugation and provided evidence against transformation as the modes of genetic transfer of F14 during transduction (Hendrickson and Duggan, 1976). Since then, it has been suggested that (a) P1 may, in some way, protect the DNA in the lysate from the action of DNase, and (b) P1 may act as a "helper" phage in the transformation of F14. These suggestions were deemed worth investigating since the calcium concentration in these transductions is 3 mM, one-tenth the optimum calcium concentration used in the transformation of *E. coli* (Cohen *et al.*, 1972).

Accordingly, we first carried out the transformation experiments under the transductional conditions and then under the optimal conditions for *E. coli* transformation (Cohen *et al.*, 1972). The DNA used was isolated from the haploid F14 donor strain, χ1254. The P1 donor used was AB1472 (*ilv*D$^-$, *met*B$^-$ and *arg*H$^-$).

The results of the first experiment (Table 2) showed that a P1 lysate prepared on χ1254 could transfer *ilv*D$^+$, *met*B$^+$, *arg*H$^+$, but not *his*$^+$ since χ1254 is *his*$^-$. P1 lysates prepared on AB1472 could transduce AB1450 for *his*$^+$ but not for *ilv*D$^+$, *met*B$^+$ or *arg*H$^+$ because both donor and recipient are mutant in these genes. When DNA from χ1254 (*ilv*D$^+$, *met*B$^+$, *arg*H$^+$) was added to the latter lysate the results did not change, indicating that P1 particles did not "help" in transformation of *ilv*D$^+$, *met*B$^+$, *arg*H$^+$. The *his*$^+$ marker could only come

Table 2. Transduction vs. transformation as the mode of transfer of the F14

Mating conditions[a]	No. of recombinants[b]/ml of markers selected			
	his$^+$	*ilv*D$^+$	*ilv*D$^+$ *met*B$^+$, *arg*H$^+$	F-*ilv*D, *met*B, *arg*H
P1·χ1254[c] x AB1450[d]	---	9.5×10^3	2.4×10^2	7.5×10^1
P1·AB1472[e] x AB1450	17.0×10^3	---	---	---
P1·AB1472 x AB1450 + 20 µg/ml DNA from χ1254 (F14)	16.0×10^3	---	---	---
20 µg/ml DNA from χ1254 (F14) + AB1450	---	---	---	---

[a] P1 lysates were added at m.o.e. = 1.

[b] The limit of detection (---) of *his*$^+$ and *ilv*D$^+$ was 10 recomb./ml and the limit of detection of *ilv*D$^+$, *met*B$^+$, *arg*H$^+$ was one recomb./ml.

[c] χ1254: *his*-1, *ilv*D$^+$, *arg*H$^+$, *met*B$^+$

[d] AB1450: *his*-1, *ilv*D, *arg*H, *met*B

[e] AB1472: *ilv*D, *arg*H, *met*B

from transduction since χ1254 has the same mutation in the histidine operon (*his*-1) as the recipient, AB1450. Transformation also did not occur when P1 was left out of the mixture. The P1·χ1254 lysate used as a control, had an F14 transductional frequency similar to that reported earlier (Hendrickson and Duggan, 1976). It can be concluded that transformation, under the experimental conditions used, does not contribute to the genetic transfer of F14.

We tested the transformability of AB1450 using the calcium treatment procedure of Cohen *et al.* (1972). AB1450 was transformed with DNA from F-prime strains (F42, F14 and F115) for plasmid linked markers at a frequency of 10^{-7}. Thus, AB1450 can be transformed, but not under the condition of P1 transduction used in these studies.

Determination of the Number of Phage Units Needed for Transduction

F14 has a molecular weight of 205 x 10^6 daltons (311 kb) (Ohtsubo, Deonier, Lee and Davidson, 1974a). This is 3.2 times larger than the transducing fragment usually packaged by P1, 64 x 10^6 daltons (Ikeda and Tomizawa, 1965 and 1968). To be packaged within P1 virions, the F14 would need to be fragmented before or during encapsidation. The F14 might then be genetically reconstructed in recipients receiving contiguous fragments from several virions. This would require at least three, but probably four, transducing particles carrying complementary fragments. Ikeda and Tomizawa (1965) estimated that there are 0.05 to 0.5% transducing particles in a lysate. With a m.o.e. of one and 10^9 p.f.u. per transduction, there were, at most, 5 x 10^6 transducing particles present in the transducing mixture.

Since the transducing efficiency for any one marker on the chromosome is approximately 10^{-4}, there are approximately 10^{5} transducing particles carrying any one segment of the chromosome. This should be true for F14 segments, but we found F14 markers to be transduced at one-half the frequency of chromosomal markers (Hendrickson and Duggan, 1976). Using the Poisson distribution equation, it was determined that the probability of one cell being infected with four random particles carrying contiguous fragments is 1×10^{-15}. The probability of being infected by three random particles carrying the F14 fragments is 4.5×10^{-12}. The probability of being transduced by three or four specific particles carrying all of F14 would be less. However, the transducing efficiency of the F14 in our lysates was 7×10^{-8}.

The requirement of multiple infection by P1 particles carrying fragments for the reconstruction of the F14 was experimentally determined by using a dose-response curve similar to the one describe by Rae and Stodolsky (1974). The results are presented as a plot of the log of the transductants versus the log of the virion concentrations in Figure 1. Transduction of F14 among the *ilv*D, *met*B, *arg*H cotransductants was determined by cross-streak matings (Hendrickson and Duggan, 1976). The slopes of the dose-response curve used to determine the mechanism for the transduction of F14 is approximately one, indicating one virion (or one unit, which will be discussed in the next section) transduced the F14. This curve was repeated several times; the slope of the curve varied from 0.85 to 1.4. The slope of the dose-response curve for the cotransduction of the F14 markers, *ilv*D, *met*B and *arg*H, is also one, indicating they are packaged together in one particle. As expected, dose-response curves

Figure 1. Nature of the F14 transduction. KF117 was grown to a concentration of 2 x 10^9 cells/ml. Selected markers were *pur*D, *ilv*D and *ilv*D, *met*B, *arg*H. The F14 transductants were determined by using a replica plate method with the *ilv*D, *met*B, *arg*H transductants onto F^- lawns. The curve is based on the Poisson distribution equation for predicting the quantitative aspects of infection:

$$(1) \qquad P(k) = \frac{e^{-m} m^k}{k!}$$

m = multiplicity of infection
k = number of particles infecting same cell
P(k) = proportion of cells infected by k transducing particles (transductants)

Taking the natural log (ln) of equation (1), it follows:

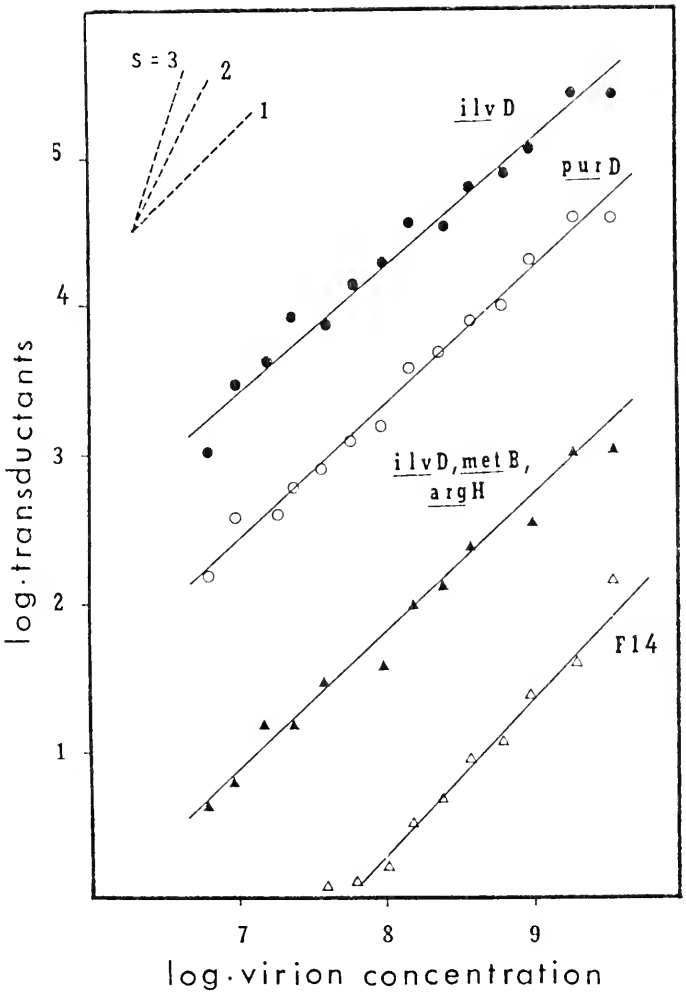
$$(2) \qquad \ln P(k) = -m + k \ln m - \ln k!$$

$-m$ and $k!$ are constants = c

$$(3) \qquad \ln P(k) = k \ln m + c$$

k = slope of curve = number particles

Dotted lines are drawn at slope (S) of 1, 2 and 3. Virion concentration is based on p.f.u./ml. Recipient concentration was kept constant, 2 x 10^9 cells/ml.


S = 3
2
1
ilv D
pur D
ilv D, met B,
arg H
F14
log·transductants
log·virion concentration
1
2
3
4
5
7
8
9

of individual markers, *ilv*D (from F14) and *pur*D (from the chromosome) have slopes indicating that their transduction involves one particle. It may be noted that *pur*D was transduced at a lower frequency than *ilv*D. This is contrary to the usual observation of a higher transducing efficiency of chromosomal markers compared to F14 markers. This may be explained by the close proximity of *pur*D to the AB1206(χ1254) chromosomal deletion, Δ(83 to 88 min), giving the transduced *pur*D fragment regions of non-homology with the non-deleted chromosomal sequence of the recipient.

The Effect of Calcium on the Transduction of F14

One interpretation of the slope of one in the dose-response curve for the transduction of F14 is that it indicates the necessity of one transductional unit; this one unit may be a single virion or an aggregate of virions. Karamata (1970) noted that the calcium concentration in phage P1 lysates caused the phage particles to aggregate. Harriman (1972) later, using prophages λ, 21 and 186 as genetic markers to study the production of P1 transducing particles in a single burst, demonstrated that transducing particles carrying different sections of the chromosome can be formed within the same bacterium. He further showed that the chance that two markers which are too far apart to be transduced will be packaged within the same cell increases with the proximity of the markers.

One could conceive that in order to transduce the large F14, aggregates may be foremed in the presence of Ca^{++} (Karamata, 1970) that consist of three or four transducing particles, produced from a single burst, carrying contiguous fragments of the F14. The particles from one of these

aggregates can then simultaneously infect the same bacterium. Once in the cell the contiguous fragments can fuse together (end to end) similar to the chromosomal fusion model of Stodolsky (1973) and form the complete F14.

If we could disaggregate the phage particles by removing Ca^{++} and other bivalent ions, there should be a loss of, or a decrease in the transducing efficiency of the lysates for the F14. Lysates of P1·χ1254 were treated with DETA (2.8×10^{-3} M) to remove Ca^{++} from the lysate in an attempt to break up aggregates of phage particles. The lysate used was treated with EDTA for three weeks, for 48 hr or for three hr prior to being tested for transductional capability. Calcium ions were added back to the usual concentration (2.8×10^{-3}M $CaCl_2$) for transduction. The lysate did not show any loss in its ability to transduce any markers or F14 when Ca^{++} was removed and readded, regardless of the period of time in EDTA (Table 3). All selected markers were transduced at their usual efficiency.

It may be asked if the chelation of Ca^{++} actually dispersed aggregated particles. Karamata (1970) noted that when he decreased the concentration of Ca^{++} by dialysis, the number of aggregates decreased to a level where they were barely detectable by ultracentrifugation in CsCl or seen in the electron microscope. Since he also noted that loss of plaqueing efficiency is correlated to the formation of aggregates, the p.f.u. should increase if phage particles disaggregate with the addition of EDTA to the lysate. The plaqueing efficiency of a P1 lysate increased as much at 200% (1×10^{10} to 3×10^{10} p.f.u./ml) and transducing efficiency of chromosomal markers increased from 10 to 60% after the lysate was treated with EDTA.

Table 3. Effect of chelation on ability of P1·χ1254 to transduce F14 into AB1450

Period lysate was treated with EDTA[a]	Transducing efficiency[b] of selected markers[c]		
	*ilv*D$^+$	*ilv*D$^+$, *met*B$^+$, *arg*H$^+$	F14[d] (F-*ilv*D, *met*B, *arg*H)
Three wk in EDTA	1.02×10^{-5}	2.0×10^{-7}	5.6×10^{-8}
48 hr in EDTA	1.11×10^{-5}	3.1×10^{-7}	7.8×10^{-8}
Three hr in EDTA	1.15×10^{-5}	2.8×10^{-7}	6.9×10^{-8}
No EDTA treatment (Control)	1.5×10^{-5}	1.6×10^{-7}	5.0×10^{-8}

[a] EDTA concentration is 2.8×10^{-3} M.

[b] No. of transductants per p.f.u. at m.o.e. = 1.

[c] Transductions were carried out with Ca^{++} added back after chelation.

[d] This was not a selected marker. It was determined by a replica plate method onto a lawn of KF104 (*ilv*D$^-$, *arg*H$^-$, *met*B$^-$ *rec*A$^-$).

These results suggest that more particles were available for infection and transduction after Ca^{++} was removed by chelation. No detectable increase in the transductional frequency of the F14 markers was noted.

These results do not support the model which suggests that the transduction of F14 occurs by means of an aggregate of transducing particles. This leaves the possibility that the one transductional unit indicated in the dose-response curve (Figure 1) could be one virion, possibly larger and more dense than the normal phage particles.

The Search for a Large and/or More Dense Phage Particle

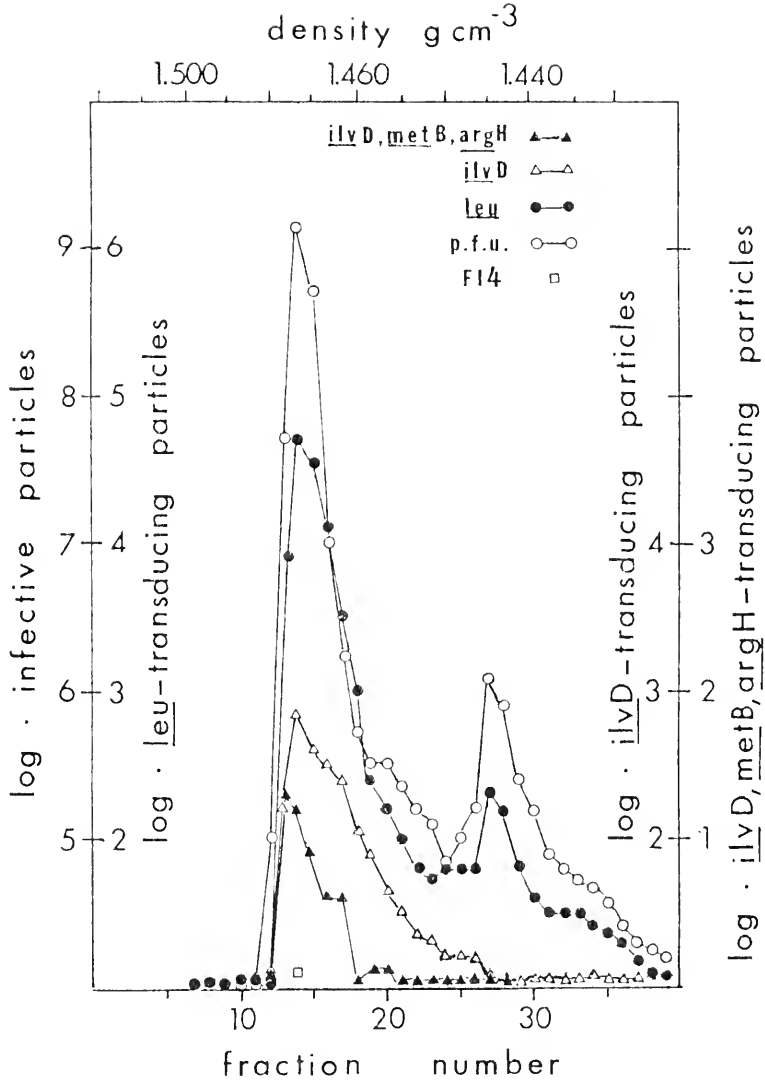
If one virion may package the entire F14, as suggested above, it should be large enough in volume to accommodate three times the DNA in normal P1 particles. Considering the density and molecular weights of head and tail proteins (Walker and Anderson, 1970) and applying the architectural principles for virus particles (Caspar and Klug, 1962), the density of such a phage particle should be greater than the density of the infectious particles.

Morphological variants have been found by Anderson and Walker (1960), Ikeda and Tomizawa (1965) and Karamata (1970); they observed two kinds of P1 particles that differed in size (density), 1.472 g cm^{-3} and 1.433 g cm^{-3}. Walker and Anderson (1970) reported four morphological variants, those reported earlier, 1.472 g cm^{-3} (a capsid diameter of 65 nm), and two newer ones with capsid diameters of 47 nm and 74 nm. Since various sized P1 particles have been seen (the larger the capsid diameter, the more dense the phage particle), it seemed worth while to look for the existence of a larger, more dense phage.

A P1 lysate made on χ1254 was subjected to buoyant density centrifugation in CsCl. After centrifugation, fractions were tested for plaque forming units (p.f.u.) and for the transducing capability of chromosomal markers (*leu*$^+$) and F14 markers (*ilv*D$^+$ only, and *ilv*D$^+$, *met*B$^+$, *arg*H$^+$, together). The *ilv*D$^+$, *met*B$^+$, *arg*H$^+$ cotransductants were tested for the F14 characteristics by replica plating onto F$^-$ lawns. The results are shown in Figure 2. We did not find a more dense phage particle in the gradient. The one transductant, in four runs of this experiment, that would transfer F14 markers was found at 1.473 g cm^{-3} (the density of p.f.u.). The transducing paricles carrying *ilv*D$^+$, *met*B$^+$ and *arg*H$^+$ appear to be a little heavier than *leu*$^+$ or *ilv*D$^+$ (0.003 g cm^{-3}).

Others have reported a loss (up to 60%) in the number of infectious and transducing particles after centrifugation in CsCl (Ting, 1962; Ikeda and Tomizawa, 1965). About 40% of the p.f.u. added to our gradients were recovered; further, only about 17% of the particles transducing *leu*$^+$, 2.2% of the particles transducing *ilv*D$^+$ and 2.0% of the particles transducing *ilv*D$^+$, *met*B$^+$, *arg*H$^+$ were recovered. Ting (1962) reported that phage P1 is not sensitive to osmotic shock when diluted from CsCl, but loses it viability when stored in CsCl. It may be that larger, more dense phage particles (if they exist) are more sensitive to CsCl——possibly even osmotically shocked. Therefore, these particles, presumably low in number (based on the transducing efficiency of F14) may never be found after being centrifuged in CsCl.

Figure 2. CsCl density centrifugation analysis of phage particles. Ten drop fractions were collected and infectious particles were determined using KF2201 as an indicator strain. *Leu*$^+$ transductants were determined with C600. *ilvD*$^+$, *metB*$^+$ and *argH*$^+$ transduction was measured with AB1450.


density g cm-3
1.500
1.460
1.440
ilvD, metB, argH
ilvD
Leu
p.f.u.
F14
log · infective particles
log · leu-transducing particles
log · ilvD-transducing particles
log · ilvD, metB, argH-transducing particles
9
8
7
6
5
4
3
2
1
10
20
30
fraction number

Discussion

We have shown that the "F14-like" transductants (Pittard and Adelberg, Bacteriol. Proc., p. 138, 1963; Hendrickson and Duggan, 1976) carry a plasmid indistinguishable from the F14 (Part I). In this study we have provided evidence supporting our contention that the mode of genetic transfer is transduction and that the transduction of F14 appears to be by one particle. Unfortunately, we have not yet found this particle.

Ohtsubo (1971) studied P1 transduction of another F-prime, F8, larger (77.4 x 10^6 daltons) than the P1 genome. Among *gal*$^+$ transductants, he found plasmids which were indistinguishable from F8. He was not successful in finding a larger or more dense particle carrying the F8. Most of the F*gal*'s were transduced in the 1.472 g cm^{-3} peak. However, he found that 100% of the *gal*$^+$ transductants from the denser region of the gradient, preceeding this peak, had received F*gal*$^+$.

Rae and Stodolsky (1974) and Rosner (1975) have reported studies on the transduction of P1 prophages carrying chromosomal genes, P1d*lac* and P1-*pro*, respectively. The prophage in each case was too large to be packaged in the single 86 nm P1 capsid. They found that the prophage was transduced by two or more particles, and complementary fragments or circular derivatives were reassembled within the transductant. Neither of them found evidence for larger or more dense particles.

The DNA in transducing particles carrying just chromosomal genes or F-prime genes differs from transducing particles carrying prophage genomes linked to chromosomal genes in their mode of replication before being packaged. The P1 prophage, upon induction, will replicate its own DNA extensively into concatemeric structures, followed by "headful" packaging of smaller linear molecules (Rosner, 1975). F-primes exist as one to two copies per cell (Jacob and Monod, 1961; Revel, 1965) and are not induced to replicate while P1 is packaging DNA (Ikeda and Tomizawa, 1965). Therefore, there will be a higher frequency of copies or partial copies of the prophage genome packaged than copies or partial copies of the F-prime genome packaged.

How does a P1 package a genome as large as F8 or F14 and transduce it at frequencies higher than multiple particle transductions? One explanation is that there exists a phage particle, which is similar to those described for phage T4 (Cummings, Chapman, Delong and Couse, 1973; Bijlenga, Aebi and Kellenberger, 1976), that has a head large enough to package the F14. The "headful" hypothesis of Streisinger *et al.* (1967) was thought to hold true for P1 since P1 DNA is terminally redundant and circularly permuted (Ozeki and Ikeda, 1968; Scott, 1968). This theory was supported by the findings that morphological variants packaged DNA molecules which are proportional to the size of their capsids (Ikeda and Tomizawa, 1965; Walker and Anderson, 1970; Karamata, 1970). Since the smaller and regular size P1 have "headfuls," it seems reasonable that there is some relationship between the cleavage of DNA and the closing of the head. However, Walker and Anderson (1970) found evidence that is at variance with the present theories of packaging DNA proposed for lambda and T4;

they found that some phage particles from each class of morphological variants of P1 were only partially filled with DNA.

Two models are generally invoked to explain the packaging of DNA into a phage head (a) the DNA is condensed into a compact body that is then surrounded by a coat of protein, (b) the empty protein shell is first assembled and subsequently filled with DNA. Lambda and T4 DNA have been shown to follow the protein shell model. Both have nucleases that cleave the DNA when a "headful" is achieved (Hohn, 1975; Uhlenhopp, Zimm and Cummings, 1974). DNA packaging in both cases is specific because one does not find T4 or lambda packaging host DNA (except when bacterial DNA is covalently linked to lambda DNA —— specialized transduction). The empty shell theory, therefore, appears to be a specific mechanism for packaging DNA in some kinds of phages.

The P1 encapsidation mechanism is not as specific as T4 in packaging DNA since about 0.5% of the P1 particles can carry bacterial DNA (Ikeda and Tomizawa, 1965; Ozeki and Ikeda, 1968). How such packaging can occur is a matter of conjecture at this time. As a proposed model, consider P1 infecting a cell, then replicating its DNA into concatemeric structures. The concatemers of DNA are then condensed into a compact form to be packaged. Capsid protein subunits are constructed around the condensing DNA, conforming to the icosahedral construction principles (Caspar and Klug, 1962). The size of the capsid formed (86 nm, 65 nm, 47 nm or some other size capsid that conforms to P1's architecture) will be determined by the amount of DNA condensed while the capsid is being formed. The rate of the DNA condensing reaction will determine the size of the

capsid formed. This must be an inherent rate since 90% of the capsids are of the 86 nm size. An endonuclease (associated with the newly formed capsid) may cleave the DNA just before this capsid is completed. This could explain full heads in the morphological variants. Partial headfuls (Anderson and Walker, 1970) may also be explained by this model. They may be formed from condensed DNA of various sizes left over from the above packaging process. The DNA may be packaged in a capsid that is indeed, large enough to package the DNA. However, the capsid, conforming to the icosahedral triangulation architecture, will carry less than 100% of its standard "headful" when it is completed.

Packaging of large plasmids by P1 may be explained by this mechanism. A plasmid that is in between stages of replication probably exists in a supercoiled state. This plasmid may already be compact enough for the capsid to condense around it, again conforming to icosahedral architecture. The other possibility is that a plasmid, being supercoiled rather than linear, may be more efficiently condensed into a more compact unit, resulting in the packaging of more DNA into any given size icosahedral capsid. In either mechanism, plasmids in a supercoiled, compact state may not be susceptible to cleavage by an endonuclease coupled to the packaging process; thus the head continues to grow until the plasmid is completely packaged. Either case may explain the transduction of F14 or F8. P1 can carry circles of DNA —— covalently closed DNA circles have been found in DNA from P1 particles (Yun and Vapnek, personal communication). Perhaps large phages were not found for the prophages, P1d*lac* or P1-*pro*, because their DNA, upon induction, form linear concatemeric structures that would produce and fill normal (86 nm) heads as linear genomes.

F-prime deletion mutants of F14 and F8 formed from P1 transduction (Pittard and Adelberg, 1963; Ramakrishnan and Adelberg, 1965; Ohtsubo, 1970; Marsh and Duggan, 1972; Lee, Ohtsubo, Deonier and Davidson, 1974), may be produced from molecules that are replicating. Under these conditions the supercoiled DNA may become relaxed, allowing it to come under the influence of the condensing process and to be cut by a proposed capsid-linked endonuclease into "headful" size DNA for packaging. This mechanism might also hold true for the encapsidation of chromosomal DNA; P1 may begin packaging at relaxed regions of the chromosome and continue encapsidating adjacent regions, explaining the observation that transducing particles for adjacent sections of the host chromosome mature within the same bacterium (Harriman, 1972). We have shown that the F14 is present in the transductants (Part I) and that the mechanism of genetic transfer is transduction. We have begun an analysis of the mechanism of transduction (one particle seems to be involved in the transduction) and have proposed a working model that encompasses our findings. The continuing study will be on the mechanism of this seemingly impossible transduction, particularly the nature of the transducing particles involved.

APPENDIX

Isolation of Plasmid DNA

The procedure used in the isolation of plasmid DNA is similar to the one described by Sharp *et al.* (1972). Bacterial cultures are grown to late logarithmic phase [approximately 10^9 cells/ml: (A_{590} = 0.85)] in two liters of L broth (56/2 medium is used for the growth of all "F14-like" strains). The cells are centrifuged at 10,000 x g for ten min and washed twice in Tris/EDTA/saline buffer, pH 8.5 (TEN) (0.05 M Tris, 0.005 M EDTA, 0.05 M NaCl) (Sharp, Hsu, Ohtsubo and Davidson, 1972). The pellet is resuspended in a spheroplast forming mixture of TEN, containing 0.1 g/ml of sucrose, 1.0 mg/ml lysozyme and 0.1 mg/ml RNase. This mixture is incubated at 37°C for ten min. The suspension is chilled in an ice bath, and the spheroplasts are lysed by adding 50 ml of 2% sodium lauryl sarcosinate solution in TEN. Spheroplasts of F14 and "F14-like" strains are lysed at 37°C and not 0°C. After several minutes a clear lysate is formed; the resulting viscous lysate is mixed and chromosomal DNA sheared by slowly forcing 50 ml batches of the lysate (30 to 45 sec) through the orifice (1.5 mm diam.) of a 50 ml disposable syringe. This is repeated three to four times. For F14 and "F14-like" plasmids this step is skipped or the DNA is gently sheared by a single slow passage (90 to 120 sec) through the 50 ml syringe. Both procedures gave similar concentrations of CCC molecules. The sheared lysate is brought to room temperature and

then the DNA is alkaline denatured by titrating the DNA mixture to pH 12.2 using 3 to 8 ml of 4 N NaOH. Since the lysate is very viscous, the NaOH does not immediately mix thoroughly into solution. Therefore, the NaOH must be added slowly to allow sufficient time for the pH meter to respond to the change in pH. To ensure adequate mixing a magnetic stirrer and teflon policeman are used. However, stirring is done gently, so as to prevent shearing of plasmid DNA. The pH is measured with a Leeds-Northrup 7400-A2 series pH meter and a Corning 476115, pH 0-14, combination electrode, after standardizing with a pH 10 buffer. The DNA solution is maintained at pH 12.2 (without stirring) for three to five min before being renatured by titrating to pH 8.5 with 2 M Tris·HCl. The principle is to denature the sheared linear chromosomal DNA and open circles, keeping the covalently closed circular DNA native. The sodium ion concentration is adjusted to 0.3 M for effective absorption of single-stranded DNA onto nitrocellulose in the next step. Excess denatured DNA is removed by using the bulk nitrocellulose method of Cohen and Miller (1969). Nitrocellulose, 100 g, (Hercules, one-fourth sec cubed) that has been ground up with a mortar and pestle and washed three times in TEN buffer, is added to the lysate and rotated 45 rev/min at a 45° angle on a New Brunswick spinnerette for one hr at 4°C. The nitrocellulose is removed by centrifugation (10,000 x g for ten min). The supernatant is filtered through glass wool to remove any suspended nitrocellulose or deris which may later interfere in dye-buoyant density centrifugation. DNA in 180 ml of the supernatant is pelleted into a saturated CsCl (at 4°C) self for 16 hr at 15 krev/min in a Beckman SW25.2 rotor. The lower 8 ml of each tube is

pooled and filtered once again through glass wool. The CsCl concentration is adjusted to a density of 1.6 g/cc. 0.5 ml of ethidium bromide (EthBr) solution (25 mg/ml) is added to give an approximate final concentration of 500 µg/ml. (EthBr did not go into solution when added as a powder to the DNA CsCl solution.) The CsCl is adjusted to a final density of 1.57 g/cc with TEN. The DNA is banded by dye buoyant density centrifugation for 36 to 48 hr at 35 krev/min in a Beckman Type 40 rotor. The lower band (covalently closed circles) were collected by dripping, pooled and rebanded for 24 hr in a Beckman Type 40 rotor. The resulting lower band is collected by dripping and stored in the dark (aluminum foil wrapped around collection tube) at $4^{o}C$. In the dye buoyant density centrifugation banding step the upper band (linear and open circle DNA) may be broad and viscous and can interfere with the collection of the lower band. This should be gently removed before collecting the lower band by using a screw type syringe attached to a microliter pipet. Ethidium bromide is extracted from the solution and the DNA by mixing equal volumes of the DNA solution with isopropanol equilibrated with saturated CsCl. The CsCl is removed by dialysis with two changed (once every two hr) of 0.25 M NaCl, 0.05 M Tris, 0.01 M EDTA, pH 8.5 at $4^{o}C$.

Electron Microscope Methods

Molecular lengths of duplexed DNA are determined by contour measurements of molecules which are spread by using the aqueous protein film method (Kleinschmidt, 1968; Davis, Simon and Davidson, 1971). The concentration of DNA in CsCl-ethidium bromide is diluted (1/10 to 1/50) in 0.5 M ammonium acetate, 0.001 M EDTA, 0.01 M Tris, pH 7.5. Cytochrome C (50 μg/ml) is added just before the DNA is spread onto a hypophase of 0.25 M ammonium acetate. Films are picked up on parlodion-coated grids (3.5% parlodion in amyl acetate), stained with 10^{-6} M uranyl acetate (in 90% ethanol) for 30 sec and washed in isopentane for ten sec. The grids are then shadowed with 80% platinum/20% palladium wire using a Balzer-Kleinbedampfungsanlage Mikro BA3 (Davis, Simon and Davidson, 1971). ColEI and F (naturally present in F14 preparations) are used as internal standards. Electron micrographs are taken using a Hitachi HU-11C or a Phillips EM201 electron microscope. Contour measurements of molecules are made by tracing projected negatives onto translucent paper and measuring the contour lengths with a Dietzgen Plan Measure or a Numonics Graphics linear calculator.

Heteroduplexing between "F14-like" plasmids and F-prime plasmids of known sequences are performed by the alkaline-formamide technique (Sharpe *et al.*, 1972). Approximately 0.1 μg of each DNA molecule to be heteroduplexed is added to double distilled water to give a final volume of 60 μl. To this mixture, 20 μl of 1 N NaOH is added. This denatures the DNA and strand separation occurs on nicked molecules. After three to five min, 20 μl of 1 M Tris·HCl and 100 μl of 0.2 M EDTA (pH 8.5) are added. The solution is dialyzed at room

temperature for two hr against 50 ml of 70% formamide in 0.25 M NaCl, 0.1 M Tris, 0.01 M EDTA, pH 8.5 (renaturation step). The formamide is used to keep single stranded DNA that are not duplexed from forming non-specific hydrogen bonds, thus giving false duplex formations. Further, formamide lowers ideal renaturation temperature (Tm-30^{o}C) to room temperature (Davis *et al.*, 1971). The cation concentration is critical in renaturation and should not exceed 0.07 M. (High salt concentration makes DNA rigid, therefore Tm is increased and the renaturation temperature is increased). The renatured solution is dialyzed against 50% formamide (seal open for less than one week) in 0.1 M Tris, 0.01 M EDTA, pH 8.5. Aqueous cytochrome C (2 mg/ml) is added to the DNA solution (just before spreading) to give a final concentration of 50 µg/ml. The final DNA solution is spread on a hypophase containing 17% formamide (fresh) in 0.1 M Tris, 0.01 M EDTA, pH 8.5. Allow DNA to "rest" on the formamide hypophase for one min before picking up with a grid. The DNA is stained and shadowed as previously described.

Heteroduplexed DNA molecules are also measured with a Numonics Graphics linear calculator. All DNA lengths are measured in kilobase-pair units (kilobases - kb) as defined by Sharp *et al.* (1972) (Bachmann *et al.*, 1976). ColEI is used as the double stranded DNA length standard (6.34 kb); and single stranded ΦX174 is used as the single stranded DNA length standard (5.375 kb) (Sharp *et al.*, 1972).

Restriction Enzyme and Gel Electrophoresis Analysis

Ethidium Bromide Extraction

The DNA samples from F14 and "F14-like" tranductants are in ethidium bromide-cesium chloride. The ethidium bromide, which is interculated between stacked base pairs, will interfer with base recognition sites of *Eco*R1. This will cause incomplete digestion and give a false restriction fragment pattern on the agarose gel (most likely a smear). Ethidium bromide is removed most efficiently from DNA by isopropanol extraction. This is done by adding an equal volume of isopropanol that has been equilibrated with saturated cesium chloride solution to an equal volume of a DNA sample. The mixture is gently agitated for one min to mix the aqueous and isopropanol fractions. The isopropanol fraction, containing extracted ethidium bromide, is removed, leaving the aqueous layer containing the DNA, behind. This procedure is repeated five times.

Ethanol Precipitation

The concentrations of F14 and "F14-like" plasmid DNA are between 10 to 20 µg/ml. The concentration of DNA samples for gel electrophoresis should be approximately 100 µg/ml so that the restriction fragment bands can be detected visually or on photographic film. To concentrate the DNA, ethanol precipitation is used. First, one-tenth volume of 5 M ammonium acetate is added to 500 µl of DNA solution. Then, three parts ethanol (100%) is added to one part DNA solution. The ethanol-DNA solution is then incubated in an ethanol-dry ice bath for ten min (or at $-20^{o}C$ for six hr). This is to dry the sample and remove salt. Next, the sample is centrifuged for ten

min at 10 krev/min. The supernatant is removed and the DNA pellet is resuspended in 100% ethanol. The DNA is again incubated in the ethanol-dry ice bath for ten min (or at -20°C for six hr) and centrifuged at 10 krev/min for 10 min. All but 0.1 ml of ethanol is removed. The remaining ethanol is removed by evaporation on a lyophilizer. The DNA pellet is redissolved in 50 µl of TE buffer (0.01 M Tris, 0.001 M EDTA, pH 7.2).

*Eco*R1 Restriction Assay

*Eco*R1 endonuclease fragmentation of the DNA samples is done using the method of Greene *et al.* (1974). The concentrated DNA solution, 50 µl, is added to 12.5 µl of 100 mM Tris·HCl (pH 7.6), 10 mM $MgCl_2$, 50 mM NaCl, forming the reaction mixture. The *Eco*R1 enzyme, 1.0 µl, (1.4×10^5 units/ml, Miles Laboratory) is added to this reaction mixture. The DNA is digested for two hr at 37°C (water bath). The reaction is stopped by adding 10 µl of 0.1 M EDTA, pH 7.6 or by freezing the mixture immediately following digestion.

Agarose Gel Electrophoresis

Agarose gel electrophoresis of *Eco*R1 fragments are determined by using the procedure of Sharp *et al.* (1973). Agarose slab gels, 15 x 13 x 0.35 cm are used as analytical gels. Agarose (0.7% and 0.5%) is melted in E buffer (0.04 M Tris, 0.02 M Na·acetate, 0.003 M EDTA, 0.18 M NaCl, pH 8.0). One percent agarose in E buffer is used to seal the bottom and sides of the 15 x 13 x 0.35 cm gel forms before the gel is poured. Gels are poured when the agarose solution is approximately 50°C. Samples are then prepared for loading onto the gel: 10 µl of DNA samples, 10 µl of E buffer,

4 µl of dye solution (0.025% bromphenol blue, 50% glycerol in E buffer). After the gels are loaded, the voltage is set at 100 V. The gels are run until the dye marker is 12 cm from the top (four to six hr). The voltage is reduced to 75 V if the gel becomes warm to touch. Heat tends to spread the bands and reduce resolution. DNA bands are visualized after staining the gels in E buffer containing ethidium bromide (2 µg/ml) for one hr. The stained bands are visualized by fluorescence over a long wavelength ultraviolet light (Sharp *et al.*, 1973). Gels are photographed using a shortwave ultraviolet light and Polaroid 57 or Polaroid 55 (p/n) film.

Polyacrylamide Gel Electrophoresis

Four percent polyacrylamide gels are used for resolving the smaller fragments of the *Eco*R1 digestion. Gels are made by adding 4 gm acrylamide, 0.2 gm N'N'-Methyline bisacrylamide, 1.0 ml of 10% NH_4 persulfate and 50 µl TEMED per 100 ml of E buffer. NH_4 persulfate and TEMED are added just before the gel is poured; these are gel accelerators. After the gel is set, the same procedures are used for analyzing *Eco*R1 fragments as were used for agarose gels. The one exception is that the gels are stopped after the dye has run 8 cm (four to six hr).

Molecular Length Determination

Molecular length of *Eco*R1 fragments were determined by plotting the Rf vs the log of the molecular length (in kilobases). The ten endonuclease *Eco*R1 fragments generated from F8(P6) and the 19 *Eco*R1 fragments generated from F are used as standards for estimating molecular lengths (kb) of other DNA species in the same gel (E. Ohtsubo, personal communication; Childs *et al.*, 1977).

Procedures for DNA Heteroduplexing and DNA Spreading Techniques[1]

Stock Solutions for DNA Spreading and Heteroduplexing - (Make all solutions with double distilled water)[2]

1. 10 x TE buffer pH 8.5 (1 M Tris and 0.1 M EDTA)--for formamide solution
2. TE buffer pH 8.5 (0.1 M Tris and 0.01 M EDTA--for DNA dilutions and aqueous spreading)
3. 99% Formamide (Malinchrodt or MC/B)
4. 2.5 M ammonium acetate, pH 7.5
5. 0.25 M ammonium acetate
6. 90% ethanol (47.5 ml 95% ethanol + 2.5 ml H_2O)
7. Acetone
8. 1 N NaOH
9. 1 M Tris·HCl
10. 0.2 M EDTA
11. 4 M NaCl
12. Cytochrome C (2 mg/ml)
13. 0.05 M uranyl acetate in 0.05 M HCl
14. Isopentane
15. 3.5% Parlodion in amyl acetate

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[1]All procedures are the same procedures or modifications of procedures found in Kleinschmidt (1968), Davis, Simon and Davidson (1971) or Sharpe *et al.* (1972). Some modifications were worked out by Drs. Eiichi Ohtsubo and Daniel Vapnek, in whose laboratories I learned these procedures.

[2]Do not filter solutions ---- filters contain wetting agents. If you must

Slide Preparation for Aqueous or Formamide Spreading

1. Wash in any lab detergent and rinse thoroughly.
2. Rinse in hot water.
3. Rinse in distilled water.
4. Put in acid bath (overnight)(H_2SO_4-chromate)
5. Rinse in distilled deionized water.
6. Dip in 0.25 M ammonium acetate (pH 7.0).
7. Five or 10 min before mounting drain slides in a beaker covered with plastic petri dish (to keep out dust).

Stain Preparation for Aqueous or Formamide Spreading - Work with uranyl salts only under the hood (slightly radioactive)!

1. Stock 0.05 M uranyl acetate in 0.05 M HCl in 95% EtOH (one can also use H_2O).
2. Store stock in a brown bottle in the dark, covered with foil.
3. Dilute stock to 10^{-6} M in 90% EtOH, 47.5 ml 95% EtOH, 2.5 M distilled water.
4. Shake. Keep in covered container under the hood.
5. Use within one hour.

Procedure for Coating Grids

Solutions

3.5% Parlodion in amyl acetate. Store in brown bottle over desiccant.

1. Wash wire mesh and chamber (or fuchner funnel) with acetone. Rinse with tap water. Wash thoroughly with detergent and hot water. Rinse

---

[2] filter, then rinse filter twice under vacuum with double distilled water. Then rinse flask with double distilled water.

with deionized water for 5 min. Clean one pair fine and one pair superfine forceps in a similar manner and leave in acetone.

2. Fill chamber (or funnel) with deionized water. Then lift the wire mesh to eliminate air bubbles and return to bottom.
3. Add 5-10 drops of the parlodion solution and let stand for 10 min. Remove film (with a clean glass pipet) to remove dust.
4. Add grids, shiny side up or down (but be consistant) to the edges of the wire mesh.
5. Add one-two drops of parlodion solution and let stand for 30 sec. Drain water from the chamber.
6. Remove wire mesh from chamber and allow to dry at 60°C for 10 min.
7. Drain wire mesh as well as possible and put into desiccator on top of filter paper. Cover mesh with plastic petri dish. Evacuate for at least 5 hr.

Solution for Heteroduplexing Procedure

A. Denaturation Step

1. 1 N NaOH
2. 1 M Tris·HCl
3. 0.2 M EDTA (pH 8.5)

B. Renaturation Step - 70% formamide solution (make just before use)

1. 5 ml 10 x TE buffer, pH 8.5 (1 M Tris + 0.1 M EDTA)
2. 3.1 ml 4 M NaCl
3. 6.9 ml doubled distilled water
4. $\frac{35 \text{ ml}}{50 \text{ ml}}$ Formamide (99%)

C. EM Preparation Step - 50% formamide solution (make just before use)

1. 5 ml 10 x TE buffer, pH 8.5
2. 20 ml Doubled distilled water
3. $\frac{25 \text{ ml}}{50 \text{ ml}}$ Formamide (99% - freshly open within the last two wk)

Procedure for Heteroduplexing DNA Molecule

1. Denaturation Step
   a. Add 0.1 µg of each molecule to a small test tube (12 mm x 74 mm or 6 mm x 50 mm). Add water to give total volume of 60 µl.
   b. Add 20 µl of 1 N NaOH. Let mixture stand for 4 to 5 min.
   c. Neutralize with 20 µl 1 M Tris·HCl, and then add 100 µl of 0.2 M EDTA (pH 8.5).
2. Renaturation Step

   Dialyze final solution from denaturation step for 2 hr at room temperature against 50 ml of the 70% formamide solution in a large screw cap test tube (25 mm x 200 mm). Rotate tube on a rotor at 16 rpm. Cover tube with aluminum foil (ethidium bromide may still be present).
3. EM Preparation Step

   Dialyse final solution from renaturation for 2 to 4 hr at $4^{o}C$ against 50 ml of the 50% formamide solution in a large screw cap tube (25 mm x 200 mm). Rotate tube on a rotor at 16 rpm.

4. EM Spreading Solution

   a. Add 30 µl from final solution from EM preparation step to a small test tube (5 mm x 75 mm or 6 mm x 50 mm).

   b. Add 10 µl of formamide (99% - freshly opened) to the tube.

   c. Next add 5 µl of reference DNA (ColEI for dsDNA, ΦX174 for ssDNA).

   d. Finally add 5 µl of cytochrome C (2 mg/ml) - just before spreading.

5. See formamide spreading procedure for hypophase needed for the 17% formamide solution below.

Solutions for Formamide Spreading

A. Hypophase Solution: Make at last minute.

   150 ml total (sufficient for 4 grids in a Teflon dish)

   For 17% formamide: 12 ml of 10 x TE buffer pH 8.5 (1.0 M Tris + 0.1 M EDTA)
   112.5 ml water (double distilled)
   25.5 ml 99% formamide

B. Spreading Solution: Mix at last minute

   For 45% formamide: 0.1 ml of 10 x TE buffer pH 8.5
   0.45 ml 99% formamide
   0.25 ml water
   0.80 ml

   Take 80 µl of the stock spreading solution and add:

   1. DNA solution in TE e.g. 10 µl of 5 µg/ml to give 0.5 µg/ml.

2. Reference DNA[1] in TE, e.g. 5 µl of 5 µg/ml to give 0.25 µg/ml.

3. Cytochrome C, e.g. 5 µl-10 µl of 1 mg/ml (or 2 mg/ml) to give 50-100 µg/ml. Add Cytochrome C last - just before spreading.

Final volume is 100 µl, sufficient for 4-6 grids (two spreads[2]). Figure out in advance the amounts of each ingredient. Water and formamide concentrations can be varied in both hypophase and spreading solution to give a variety of concentrations (60/30; 45/17; 30/10 for example)

Shake spreading solution and load 50 µl into a 50 µl Hamilton syringe (air-tight) fitted with about 8 inches of intramedic tubing. Don't introduce sample into syringe (saves washing). One can also use 50 µl micropipettes with an Adams screw-type suction apparatus.

Solutions for Aqueous Spreading and Mounting

Hypophase 0.25 M ammonium acetate pH 7.5

Spreading solution:

1. 20 µl of 2.5 M ammonium acetate
2. 10 µl of TE buffer pH 8.5 (0.1 M Tris + 0.01 M EDTA)
3. 0.5 to 1 µg DNA (sample)
4. 0.2 µg of reference DNA (if needed)
5. Water to 95 µl
6. 5 µl cytochrome C (add just before spreading)

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[1]Reference DNA: ColEI for d.s. circles; ΦX174 for s.s. circles.

[2]A "Spread" is defined as a volume of DNA solution that is run down slide ramp and spread on surface of the hypophase.

Technique for Aqueous and Formamide Spreading

1. Fill plastic petri dish (or Teflon dish)[1] with hypophase and sweep surface twice with plexiglass rods to remove dust.
2. Lay clean slide (previously dryed in ammonium acetate atmosphere) in dish and prop against one of the plexiglass rods.
3. Apply sample slowly (back and forth across the slide) within about 1 cm of the hypophase/slide interface. A steady slow, but smooth, delivery is most necessary.
4. If using formamide let DNA "rest" for about one min after finishing the spread.
5. Pick up sample using clean forceps with grid, film side down, by touching surface from above quickly about one grid distance from the glass/hypophase interface. Stain 30 sec in uranyl acetate, agitating constantly. Then dip 10 sec in 2 methyl butane (isopentane). A second sample should be taken at the glass/hypophase interphase.
6. Put grids in covered plastic petri dish on a piece of filter paper. LABEL CAREFULLY.
7. When fully dry, transfer the grids to a narrow piece of double stick scotch tape on a glass slide; attach filter paper, labelled, with double stick tape to the bottom of the slide.
8. Next, shadow grids.

[1] Previously washed with hot detergent, rinse in hot water, rinse 5 min in deionized water. Don't touch inside after clean.

Shadowing

1. Wear plastic gloves. Minimize dirt.
2. Using the minimum amount of double stick scotch tape possible, attach grids to glass slide or covership. Attach glass slide to piece of filter paper slightly larger than glass with double stick tape.
3. Tape glass slide to the rotating shaft of the shadowing apparatus.
4. Position electrodes 10-13 cm from grids at 5-20^{o} angle (1 cm rise for every 10 cm run).
5. Electrode: use a 5 cm (10 cm in U of F's Balzer-Kleinbedampfungsanlage Mikro BA3) tungsten wire bridge between the electrodes (v-shaped) and tightly wrap a 4 cm piece of 80% Platinum/20% Palladium (Pt/Pd) wire in the center of the tungsten wire.
6. Rotate grids at 45 rpm.
7. Turn on electrodes and increase amperage until Pt/Pd melts (about 22 amps on Ladd evaporator or about 4 on the Balzer scale). Hold there for 30 sec. Then increase amperage through electrode until wire glows white-hot (about 27-30 amp, 5-8 on Balzer scale -- units unknown). Shadow until the platinum/Pd wire is gone (about 10 min).
8. Remove grids; check filter paper to ensure that shadowing worked.

Printing of Electron Micrographs

1. Make a transparent positive of the original electron micrograph negative on Kodalith ortho film, type 3 (graphics arts film-Kodak). Develop transparancy with full strength Selectol (Kodak). Use a black background for printing.
2. Make another negative by sandwiching the emulsion side of the above positive with the emulsion side of another sheet of Kodalith ortho film. Again develop the film with full strength Selectol. Use a black background for printing.
3. Make a print from new negative using polycontrast paper.

List of Equipment Needed

1. Falcon Plastics

   No. 4021 LabTek plastic petri dishes Square 100 x 15 mm

   No. 2054 Plastic test tubes with cap 12 x 75 mm
2. Wire mesh to fit buchner funnel or drainage chamber
3. Buchner funnel ≥8 inch diameter (or drainage chamber)
4. Grids - 300 mesh with handles from Ted Pella Co., P.O.B. 510, Tustin, CA 92680 or 200 mesh Athene Type Grids Type H Cat. 7576C Polysciences, Inc.
5. Shop

   Teflon dish (10 mm deep x 80 mm long x 40 mm wide)

   Two plexiglass square rods (10 mm on a side x 150 mm long)
6. Clay Adams

   Intramedic tubing polyethylene tubing, Cat no. PE 50 (7411)

   I.D. 0.023" O.D. 0.28" x 100 feet.

   Hamilton 50 µl airtight syringe

7. 80% Pl/20% Pd Wire

   Ernest F. Fullam, P.O. Box 444, Schnectady, N.Y. 12301

8. Formamide

   99% Malinchrodt

9. Tungsten Wire - 0.025"

10. Culture tubes

    Kimax brand 45066-A 25 mm x 200 mm

    Dispo T1338-1 12 mm x 75 mm

    Corning 9820 6 x 50 mm

LITERATURE CITED

Adelberg, E.A., and S.N. Burns. 1960. Genetic variation in the sex factor of *Escherichia coli*. J. Bacteriol. *79*:321-330.

Anderson, T.F., and D.H. Walker. 1960. Morphological variants of bacteriophage P1. Science *132*:1488.

Bachmann, B.J., K.B. Low and A.L. Taylor. 1976. Recalibrated linkage map of *E. coli* K12. Bacteriol. Rev. *40*:116-167.

Bijlenga, R.K.L., U. Aebi and E. Kellenberger. 1976. Properties and structure of a gene 24-controlled T4 giant phage. J. Mol. Biol. *103*:469-498.

Caspar, D.L.D., and A. Klug. 1962. Physical principles in the contruction of regular viruses. Cold Spring Harbor Symp. Quant. Biol. *27*:1-24.

Childs, G.J., H. Ohtsubo, E. Ohtsubo, F. Sonnenberg and M. Freundlich. 1977. Restriction endonuclease mapping of the *E. coli* K12 chromosome in the vicinity of the *ilv* genes. J. Mol. Biol. *in press*.

Cohen, S.N., and C.A. Miller. 1969. Multiple molecular species of circular R factor DNA isolated from *E. coli*. Nature *224*:1273-1277.

Cohen, S.N., A.C.Y. Chang and L. Hsu. 1972. Nonchromosomal antibiotic resistance in bacteria: genetic transformation of *E. coli* by R factor DNA. Proc. Natl. Acad. Sci. U.S.A. *69*:2110-2114.

Cummings, D.J., V.A. Chapman, S.S. Delong and N.L. Couse. 1973. Structure aberrations in T-even bacteriophage. III. Induction of "lollipops" and their partial characterization. Virology *54*:245-261.

Davidson, N., R.C. Deonier, S. Hu and E. Ohtsubo. 1975. Electron microscope heteroduplex studies of sequence relations among plasmids of *E. coli*. X. Deoxyribonucleic acid sequence organization of F and and F-primes, and the sequences involved in Hfr formation. Microbiology *1*:56-65.

Davis, R.W., N. Simon and N. Davidson. 1971. Electron microscope heteroduplex method for mapping regions of base sequence homology in nucleic acids. *In* L. Grossman and K. Moldave (ed.), Methods in Enzymology, Vol. 21D. P. 415. Academic Press Inc., New York.

Deonier, R.C., E Ohtsubo, H.J. Lee and N. Davidson. 1974. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. VII. Mapping the ribosomal RNA genes of plasmid F14. J. Mol. Biol. *89*:619-629.

Devine, E.A., M.C. Moran, P.J. Jederlinic, A.J. Mazaitis and H.J. Vogel. 1977. *Eco*R1 cleavage sites in the *arg*ECBH region of the *Escherichia coli* chromosome. J. Bacteriol. *129*:1072-1077.

Glansdorf, N. 1967. Pseudoinversion in the chromosome of *Escherichia coli* K12. Genetics *55*:49-61.

Greene, P.J., M.C. Betlach, H.M. Goodman and H.W. Boyer. 1974. DNA replication, p. 87-111. *In* R.B. Wickner (ed.), Methods in Molecular Biology, vol. 7. Marcel Dekker, Inc., New York.

Harriman, P. 1972. A single-burst analysis of the production of P1 infectious and transducing particles. Virology *48*:595-600.

Hedgepeth, J., H.M. Goodman and H.W. Boyer. 1972. DNA nucleotide sequence restricted by the R1 endonuclease. Proc. Natl. Acad. Sci. U.S.A. *69*:3623-3627.

Hendrickson, E.R., and D.E. Duggan. 1976. Novel genotypes among transductants made with bacteriophage P1 lysates from an F14 merogenote strain *Escherichia coli* K12. J. Bacteriol. *127*:392-405.

Hohn, B. 1975. DNA as substrate for packaging into phage lambda *in vitro*. J. Mol. Biol. *98*:93-106.

Ikeda, H., and J. Tomizawa. 1965. Transducing fragments in generalized transduction by phage P1. I. Molecular origin of the fragments. J. Mol. Biol. *14*:85-109.

Ikeda, H., and J. Tomizawa. 1968. Prophage P1, an extrachromosomal replication unit. Cold Spring Harbor Symp. Quant. Biol. *33*:791-798.

Jacob, F., and J. Monod. 1961. Genetic regulatory mechanisms in the synthesis of proteins. J. Mol. Biol. *3*:318-356.

Kaiser, A.D., and D.S. Hogness. 1960. The transformation of *Escherichia coli* with deoxyribonucleic acid isolated from bacteriophage λdg. J. Mol. Biol. *2*:392.

Karamata, D. 1970. Multiple density classes of phage P1 due to tetramer formation. Mol. Gen. Genet. *107*:243-255.

Kleinschmidt, A.K. 1968. Monolayer technique in electron microscopy of nucleic acid molecules. *In* L. Grossman and K. Moldave (ed.), Methods in Enzymology, vol. 12B. p. 125. Academic Press, Inc., New York.

Lee, H.J., E. Ohtsubo, R.C. Deonier and N. Davidson. 1974. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. V. *ilv*$^+$ deletion mutants of F14. J. Mol. Biol. *89*:565-584.

Lennox, E.S. 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology *1*:190-206.

Luria, S.E., and J.W. Burrows. 1957. Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. *74*:461-476.

Marsh, N.J., and D.E. Duggan. 1972. Ordering of mutant sites in the isoleucine-valine genes of *Escherichia coli* by use of merogenotes derived from F14: A new procedure for fine structure mapping. J. Bacteriol. *109*:730-740.

Ohtsubo, E. 1970. Transfer defective mutants of sex factors in *E. coli*. II. Deletion mutants of an F-prime and deletion mapping of cistrons involved in genetic transfer. Genetics *64*:189-197.

Ohtsubo, E. 1971. Studies on sex factors in *Escherichia coli*. Ph.D. Dissertation. Osaka University, Toyonaka, Osaka, Japan.

Ohtsubo, E., R.C. Deonier, H.J. Lee and N. Davidson. 1974a. Electron microscopic heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. IV. The sequence in F14. J. Mol. Biol. *89*:565-584.

Ohtsubo, E., H.J. Lee, R.C. Deonier and N. Davidson. 1974b. Electron microscopic heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. VI. Mapping of F14 sequences homologous to ϕ80d*met*BJF and ϕ80d*arg*ECBH bacteriophages. J. Mol. Biol. *89*:565-584.

Ohtsubo, E., L. Soll, R. Deonier, H.J. Lee and N. Davidson. 1974c. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. VIII. The structure of bacteriophage ϕ80$_3$*ilv*$^+$ *su*$^{+7}$, including the mapping of the ribosomal RNA genes. J. Mol. Biol. *89*:631-646.

Ozeki, H., and H. Ikeda. 1968. Transduction mechanisms. Ann. Rev. Genetics *2*:248-278.

Palchaudhuri, S., W.K. Maas and E. Ohtsubo. 1976. Fusion of two F-prime factors in *Escherichia coli* studied by electron microscope heteroduplex analysis. Mol. Gen. Genet. *146*:215-231.

Pittard, J., and E.A. Adelberg. 1963. Gene transfer by F' strains of *Escherichia coli* K12. II. Interaction between F-merogenote and chromosome during transfer. J. Bacteriol. *85*:1402-1408.

Pittard, J., and T. Ramakrishnan. 1964. Gene transfer by F' strains of *E. coli* K12. IV. Effect of a chromosomal deletion on chromosomal transfer. J. Bacteriol. *88*:367-373.

Pittard, J., J.S. Louitt and E.A. Adelberg. 1963. Gene transfer by F' strains of *Escherichia coli* K12. I. Delay of initiation of chromosome transfer. J. Bacteriol. *85*:1394-1401.

Rae, N.E., and M. Stodolsky. 1974. Chromosomal breakage, fusion and reconstruction during P1dl transduction. Virology *58*:32-54.

Ramakrishnan, T., and E.A. Adelberg. 1965. Regulatory mechanisms in the biosynthesis of isoleucine and valine. III. Map order of the structural genes and operator genes. J. Bacteriol. *89*:661-664.

Revel, H. 1965. Synthesis of -galactocidase after F-duction of *lac*$^+$ into *E. coli* J. Mol. Biol. *11*:23-34.

Rosner, J.L. 1975. Specialized transduction of *pro* gene by coliphage P1: Structure of a partly diploid P1-*pro* prophage. Virology *66*:42-55.

Scott, J.R. 1968. Genetic studies on bacteriophage P1. Virology *36*:564-574.

Sharp, P.A., B. Snyder and J. Sambrook. 1973. Characterization class II restriction endonucleases from *Haemophilus parainfluenza* (HpaI and HpaII). Biochemistry *12*:3055-3063.

Sharp, P.A., M. Hsu, E. Ohtsubo and N. Davidson. 1972. Electron microscope heteroduplex studies of sequence relations among plasmids of *Escherichia coli*. I. Structure of F-prime factors. J. Mol. Biol. *71*:471-487.

Skurry, R.A., H. Nagaish and A.J. Clark. 1976. Molecular cloning of DNA from F sex factors of *E. coli* K12. Proc. Natl. Acad. Sci. U.S.A. *73*:64-68.

Stodolsky, M. 1973. Chromosome fusion. Genetics *73*:65-66.

Streisinger, G., J. Emrich and M.M. Stahl. 1967. Chromosome structure in phage T4. III. Terminal redundance and length determination. Proc. Natl. Acad. Sci. U.S.A. *57*:292-295.

Swanstrom, M. and M.H. Adams. 1951. Agar layer method for production of high titer phage stocks. Proc. Soc. Exp. Biol. Med. *78*:372-375.

Ting, R.C. 1962. The specific gravity of transducing particles of bacteriophage P1. Virology *16*:115-121.

Uhlenhopp, E.L., B.H. Zimm and D.J. Cummings. 1974. Structural aberrations in the infection of *E. coli* with phage T4. J. Virol. *13*:548-550.

Walker, D.H., and T.F. Anderson. 1970. Morphology variants of coliphage P1. J. Virol. 5:765-782.

BIOGRAPHICAL SKETCH

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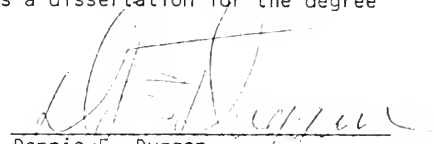

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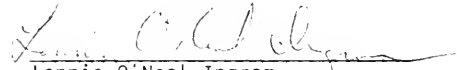

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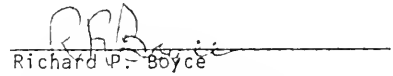

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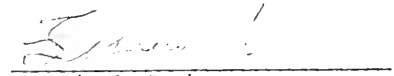

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This dissertation was submitted to the Graduate Faculty of the Department of Microbiology and Cell Science in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor or Philosophy.

July, 1977

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Dean, Graduate School